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BIOLOGICAL BULLETIN

STUDIES ON THE RELATION BETWEEN AMITOSIS AND MITOSIS.

IV. Nuclear Division in the Somatic Structures of the Proglottids of Moniezia.

V. General Discussion and Conclusions Concerning Amitosis and Mitosis in Moniezia.

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IV. Nuclear Division in the Somatic Structures of the Proglottid of Moniezia.

1. *The Genital Ducts.*

The various genital ducts of the cestode proglottid appear to arise by the division and differentiation of the parenchymal cells, if the syncytium which composes the parenchyma can properly be said to be composed of cells.

Moreover, this division and differentiation apparently occurs *in situ*, at least to a large extent, and not by outgrowth of the cells from a particular region. As was noted in the first paper of this series the development of the reproductive organs begins, or at least first becomes visible, in a region near the longitudinal nephridial canals and the lateral nerve cords, *i. e.*, midway between the region where the ovaries will appear and the lateral border of the proglottid (Child, '07*a*, Figs. 1*a*, 1*b*), the first portion to appear being parts of the ducts. The earliest indication of development is an increase in the number of the parenchymal nuclei in this region (Child, '07*a*, Figs. 2 and 3). The area of proliferation gradually extends in both directions and the "cells"

differentiate into the ducts and terminal organs. The development of the ducts does not appear to be an outgrowth from the region first involved, but seems rather to be due to an extension of certain stimuli or conditions in the parenchyma which bring about proliferation and gradual differentiation of all cells which lie within the region affected. I have found it impossible to reach any other conclusion regarding the development of these ducts. Moreover, in the earliest stage of the ducts I have never observed a single case of mitosis or anything resembling it. On the other hand Figs. 2 and 3 of my earlier paper (Child, '07*a*) show that amitoses are frequent. In the early stages there is a marked difference in size of the nuclei in the central and peripheral portions of the area in which proliferation is occurring. This is very clearly shown in Fig. 2 (Child, '07*a*). Here the nuclei about the periphery of the proliferating region are of the same size as the nuclei elsewhere in the parenchyma, but toward the center their size decreases until they are only a small fraction of the size of the parenchymal nuclei in general. Evidently proliferation is much more rapid in the central than in the peripheral regions of the proliferating area.

In somewhat later stages the rapidity of division apparently decreases and the nuclei of the central regions gradually increase in size until they are almost or quite as large as those about the periphery. At this time the ducts are visible as bands or cords of very numerous nuclei each surrounded by small areas of cytoplasm. Between the ducts and other portions of the parenchyma, however, no limiting membrane exists nor do the "cells" appear appreciably different in character from those composing the parenchyma.

From this stage on the differentiation of the walls of the ducts gradually takes place; muscle-fibers develop, a lumen appears, and nuclear division becomes less and less frequent.

In Figs. 1–5 (Plates VIII. and IX.) typical portions of the duct-regions before differentiation has begun are shown. Fig. 1 (Pl. VIII.) is taken from the lateral end of the developing ducts at a time when the proliferation has extended to a point midway between the longitudinal nephridial canals and the lateral margin of the proglottid. From right to left the figure includes the whole width

of the proliferating area from which this portion of the ducts will develop. The axis of the developing duct corresponds to a line drawn vertically through the middle of the figure. The nuclei are just beginning to be affected by the conditions which produce proliferation ; thus the figure represents approximately the earliest stage at which this portion of the ducts is distinctly visible. From the cord of cells in this lateral region both vagina and vas deferens differentiate, but there is no distinction between them until much later stages.

Fig. 2 (Pl. VIII.) represents a section similar to Fig. 1 of the cord of cells from the same region at a somewhat later stage. The nuclei are more numerous and amitoses are apparently more frequent. At the right is one of the very rare cases of mitosis observed in the development of the genital ducts. It will be observed that it lies on the border of the region involved in proliferation and the same is true of most other cases observed. Not a single case of mitosis has been observed in the axial region of the cell-cords from which the ducts arise and only six cases of mitosis have ever been seen outside the ovaries and testes in *Moniezia expansa* and *M. planissima*, though extended search for them has been made.

Fig. 3 (Pl. VIII.) is a section similar to Fig. 1 of the cord of cells from which the vas deferens develops, taken from a point near its upper or inner end where it does not adjoin the vagina. As regards nuclear division this figure is very similar to the preceding. On the right is shown one of the cases mentioned and figured in earlier papers (Child, '07*a*, '07*b*, '07*d*) where the two parts of a nucleus which is apparently undergoing amitosis stain differently.

In Fig. 4 (Pl. IX.) a transverse section of the vas deferens at a somewhat later stage after the lumen has appeared is shown. Amitotic nuclear divisions are still taking place, but apparently less rapidly than in earlier stages, or else the nuclei increase in size more rapidly between successive divisions.

Fig. 5 shows a portion of a vas efferens near its junction with others. Two cases of undoubted amitosis are visible. Fig. 6 is from the outer portion of the wall of the oviduct and includes one case of mitosis besides several amitoses.

The preceding figures are sufficient to indicate the general type of development of the genital ducts as regards division. Since we are concerned with the cytology rather than with the morphology, it is unnecessary to follow the development of the structures step by step, for the later stages show nothing of importance cytologically, which cannot be observed in the earlier stages. Amitosis continues to be the characteristic method of nuclear division throughout the development.

2. *Other Proglottidal Structures.*

The remaining figures (Figs. 7–19) are selected as examples from a large number of camera drawings of parenchymal structures.

Fig. 7 (Pl. IX.) probably represents one of the smaller nephridial ducts at an early stage in transverse section. One very clear case of amitosis and two other probable cases are visible.

Fig. 8 (Pl. IX.) and Fig. 9 (Pl. X.) are taken from the region of the lateral longitudinal nerve-cords in the growing region just posterior to the scolex. In this part of the body the cytoplasmic areas about the nuclei are more extensive than further posteriorly.

Figs. 10–14 (Pl. X.) show cells and syncytial masses from the general parenchyma in the region where the proglottidal boundaries are just becoming visible, *i. e.*, in a region of rapid proliferation of the parenchymal nuclei. Not a single case of mitosis has ever been observed in this region.

Two cells from the interproglottidal glands are shown in Figs. 15 and 16 (Pl. X.) at a stage where differentiation is advanced. In early stages the regions where these glands appear are not visibly different from other regions. The first visible indication of gland-development is the formation by amitotic proliferation of groups of nuclei along the interproglottidal boundary. Each of these groups gives rise to a considerable number of elongated unicellular glands like those in the figures, arranged radially about a common outlet. Amitosis is much more frequent before differentiation occurs, but as Figs. 15 and 16 indicate, often occurs after differentiation is somewhat advanced. The figures show the earliest visible stages in the formation of the secretory product. The cytoplasm gradually becomes filled with deeply staining, granular masses.

Cuticle-forming cells of the external layer of the body are shown in Figs. 17–19. These cases are taken from the region where proglottids are beginning to form: in this region division is more frequent in these cells than in proglottids advanced in development.

It is perhaps unnecessary to repeat that these figures are merely a few cases selected almost at random from among my camera-drawings. The number of figures might be increased almost indefinitely if desirable, but I think these are sufficient to show that amitosis is a typical feature of development in *Moniezia* both in the germ cells and in the soma.

Unfortunately it is at present impossible to complete my observations by examination of the stages between the embryo and the adult tape-worm, for the life-history and intermediate host of the genus are unknown.

V. General Discussion and Conclusions Concerning Amitosis and Mitosis in Moniezia.

Before any general interpretation of the facts concerning the rôle and frequency of the two forms of division is possible it is necessary to know something regarding the physiological conditions which determine the occurrence of each of the two methods of division. At present, however, we have no real knowledge regarding these conditions and are limited to hypotheses and surmises. In a recent paper (Child, '07*c*) I have made certain suggestions along this line to which attention may be called here.

It was pointed out in the paper referred to that the stimulus to nuclear division and growth is apparently not identical with the presence of an excess of nutritive material, for it is a well known fact that extensive regeneration will occur in many forms, *e. g.*, *Planaria*, even after reduction of the body by long continued starvation, to a fraction of the original size. In such cases the regenerating region derives its nutritive material from other regions of the body, which consequently decrease in size more rapidly than in individuals where no regeneration is taking place. Evidently in such cases the nutritive material goes to the regenerating region at the expense of other parts because the demand is greater there than elsewhere. In short, conditions exist in this

region in consequence of which it deprives other regions of material. Doubtless these conditions are largely chemical in nature.

The existence of such conditions in this and other similar cases justifies the conclusion that the stimulus to cell-division and growth is not identical with the presence of excess of nutritive material. Admitting the existence of a stimulus to division independent of the presence or absence of nutritive material we may expect to find conditions different within the cell or nucleus, according as the changes which occur in consequence of the stimulus are or are not balanced by the intake of material. In case they are balanced, a condition of equilibrium is more or less perfectly maintained or else changes in both directions from the condition of equilibrium alternate more or less rhythmically. In consequence of the complex character of the cell and the more or less central position of the nucleus, the occurrence of rhythmical cyclical changes is to be expected rather than the maintenance of a condition of absolute equilibrium.

If on the other hand the demands of the nucleus are not met by the intake of material the condition of equilibrium is not attained, and the processes in the cell, so far as this point is concerned, are not cyclical, but acyclical or orthodromic.

Now the nuclear phenomena which occur in connection with mitosis are very clearly cyclical; the condensation of the chromatin and the disappearance of the nuclear membrane are followed by an apparent reversal which terminates in the reconstitution of a nucleus and the resolution and distribution of the chromatin.

In amitosis, on the other hand, no such cyclical changes occur. The nuclear structure remains the same throughout the whole process. There is no disappearance or transformation, followed by return to the original condition, of any part. Apparently the process consists essentially in increase in size in consequence of formation of new nuclear material, followed by separation into physiologically independent parts. Nothing in the visible phenomena indicates the occurrence of reversal in direction of the processes involved. Division itself may be due either to physical factors or to the establishment with increasing size of more or less independent regions or centers of activity. The appearance

of new nucleoli in widely separated regions of the nucleus and the difference in tingibility of the two parts of a dividing nucleus, which are often observed in *Moniezia*, indicate the existence of a certain degree of physiological independence before separation of the parts.

If this interpretation of the visible phenomena is correct it appears probable that mitosis is associated with certain cyclical processes, in the nucleus and amitosis with acyclical or orthodromic processes. In other words, in order to divide mitotically the nucleus must be in a condition approximating equilibrium between intake of material and functional transformation. If the stimulus to growth is so strong that the nucleus is forced far from a condition of equilibrium amitotic division may occur. Of course in all cases where the demand exceeds the supply the actual rate of growth or division is determined not by the intensity of the stimulus but by material available. According to my suggestions, it is not rapidity of growth or division which determines or influences the form of division, but rather the relation between the stimulus to growth and the intake of material. Division itself is probably an incidental result of growth. These suggestions are of course merely provisional, being scarcely more than surmises, but they may perhaps serve as a working hypothesis for future investigation of the problem.

But whatever value the future may assign to these suggestions, the facts now known regarding the occurrence of amitosis seem to be in accord with this hypothesis, as will be clear from a brief consideration.[1] Most of the earlier observers agree in the conclusion that amitosis is characteristic of regions of extreme physiological activity connected with assimilation, secretion, etc. (Ziegler, '91 ; Ziegler and Vom Rath, '91 ; Vom Rath, '95, etc.). More recent observations of others and myself some of which are briefly mentioned in my earlier paper (Child, '07*c*) show that amitosis is of frequent occurrence in regulatory growth, which is much more rapid than normal growth. It is also the typical form of division in the imaginal organogeny of certain diptera and very probably of many insects. Here, likewise, the growth is

[1] More extended discussion of the bibliography is postponed until additional data have been recorded.

exceedingly rapid. In certain vertebrate embryos amitosis is apparently much more frequent in the more rapidly growing than in the less rapidly growing regions. In certain forms, such as trematodes and polychætes, which rapidly produce a very large number of generative cells, amitosis appears to be the characteristic method of division in the primitive germ-cells.

All of these cases concern regions of extreme assimilative activity and there are certain indications that, so far as the individual nuclei are concerned, they are regions which are far from equilibrium. In all such regions, for example, the cytoplasm is relatively small in amount, *i. e.*, the nuclei are relatively much more numerous than in less rapidly growing regions; secondly the nuclei in these regions are usually much smaller than in less rapidly growing regions. Often there are differences in this respect between the peripheral and central portions of such regions, the central portions showing more extreme conditions than the peripheral. Evidently the nuclei in these regions do not attain a condition of equilibrium but are forced on in a given direction by some stimulus as rapidly as the material available will permit. According to the hypothesis set forth above these are exactly the conditions in which amitosis may be expected to occur.

But according to this hypothesis also amitosis is not necessarily confined to regions of rapid growth. If little material is available actual growth may be exceedingly slow. Moreover, it is possible that nutritive material may be present outside the cell or even in the cytoplasm, in excess but in consequence of scarcity of cytoplasm or other special conditions may become available for the nucleus only very slowly or not at all. In such cases amitosis might occur in the presence of an apparent excess of nutritive material. Possibly various cases of amitosis observed in nuclei lying in the yolk of meroblastic eggs and surrounded by only a small amount of cytoplasm may be cases of this kind.

And again it is possible that the differentiation of the cytoplasm in certain directions may bring about conditions favoring amitosis; in such cases the amount of undifferentiated cytoplasm may be insufficient to maintain the nucleus in equilibrium. Thus amitosis may be expected, and has often been found in highly differentiated cells.

Doubtless various other conditions may arise in the cell which favor amitosis, but the above consideration is sufficient to indicate the relation between the facts and the hypothesis.

The opinion of Ziegler and Vom Rath (Ziegler, '91 ; Ziegler, and Vom Rath, '91 ; Vom Rath, '95) and many later observers, that amitosis is never followed by mitosis, but always leads to degeneration and death is without doubt incorrect. But that degeneration and death should follow amitosis in certain cases is to be expected, if my hypothesis is correct. Under extreme conditions the nucleus or cell many be forced so far from equilibrium that changes occur which render return impossible, and degeneration and death follow. In regions of rapid embryonic growth degenerating nuclei are not infrequently found, but in these, as in other cases, the degeneration is not a necessary consequence of the amitosis, but both are merely indications of a physiological condition, which in many cases brings about amitosis without degeneration, but in extreme cases produces degeneration. Nuclear fragmentation is a frequent accompaniment of degeneration, but even in these cases the physiological conditions may be in general similar to those occurring in normal amitosis.

Returning now to the case of *Moniezia*, the facts concerning the distribution of relative frequency of amitosis and mitosis are briefly as follows. As regards the germ cells, amitosis is much more frequent than mitosis during the development of ovaries and testes (Child, '07*a*, '07*b*). Mitoses are of very rare occurrence during the earlier stages of development, but in some chains and proglottids and gonads appear somewhat more frequently in later stages before the growth-period. This developmental period characterized by amitosis is followed by the growth-period, at the beginning of which a spireme appears, and later by maturation. In the female cells maturation exhibits the features typical of the process in other species (Child, '07*a*): in the male cells typical maturation occurs, but in addition to this a peculiar process of fragmentation of the nuclei of the first spermatocytes is of common occurrence (Child, '07*b*), and apparently results in the formation of nuclei indistinguishable from the spermatid-nuclei formed in the typical manner. Whether these nuclei actually give rise to spermatozoa or not cannot be determined with cer-

tainty, but the observations seem to indicate that they may. The relative frequency of the two processes, typical maturation and fragmentation, appears to vary in different chains, proglottids, and regions. In some chains typical maturation has been observed only rarely, and fragmentation very frequently in the testes, yet these chains apparently produce as many spermatozoa as others; in some proglottids of certain chains the maturation-phenomena are similarly very infrequent while fragmentation is of common occurrence, while in other proglottids typical maturation is more frequent, and finally, in many proglottids the maturation-phenomena have been observed much more frequently in those testes which occupy the lateral regions of the proglottid, while fragmentation appears to be more characteristic of the testes in the middle regions.

The first cleavage of the egg is at least usually, if not always mitotic, but in later stages amitosis becomes the characteristic method of division, mitosis appearing only occasionally, and then in the larger nuclei (Child, '07*d*). Throughout the stages of cleavage observed nuclear division is far in advance of cytoplasmic division.

In the development of somatic structures in the proglottid mitosis is almost never seen, amitosis being the typical method of division. When mitoses occur they occur as isolated cases, usually at or near the periphery of regions of proliferation.

If my observations are correct, amitosis is the more common method of division in the generative cycle, except during the period of maturation and early cleavage. In the somatic cells of the adult body it appears to be the usual method at all times. Later embryonic stages inhabiting the intermediate host are not at present known, but conditions will probably be found to be similar in these.

Considering these facts in the light of the hypothesis presented above, we find them in general in accord. In the first place *Moniezia* produces rapidly an enormous number of generative cells and this involves a very large amount of assimilation. These, according to our hypothesis, are conditions favorable to the occurrence of amitosis, and we have found that amitosis is the characteristic method of division in the development of ovaries and testes.

But even in this period mitoses occasionally occur, their relative frequency differing in different chains, proglottids and regions. These differences may be due to local differences in nutrition; doubtless different chains and often different proglottids, receive different amounts of nutritive material, and in those best nourished, some of the nuclei may attain equilibrium occasionally. The fact that mitoses were more frequently observed near the lateral borders than in the middle of the proglottid may be due to similar differences in condition. Near the lateral border the proliferating regions — chiefly testes — are less numerous than in the middle regions and the absorptive surface is relatively greater hence more nutritive material may be available for each, and conditions permitting mitosis may be more frequently attained than elsewhere.

Moreover, mitoses in the developing testes and ovaries seem to occur more frequently in the later stages during the last division preceding the growth period, than in earlier stages. It is probable that in these stages the stimulus to growth is not as great as in earlier stages and some of the nuclei attain a condition of equilibrium.

It is by no means certain that the peculiar process of fragmentation of the spermatocyte-nuclei (Child, '07*b*) is to be regarded as due to the same conditions as other cases of amitosis. On the other hand, there can be little doubt that the conditions which, according to the hypothesis, favor amitosis are present in the testes. The development of each testis involves the formation of a relatively enormous amount of nuclear and cytoplasmic material and each prologottid contains hundreds of testes. Failure to attain equilibrium might be expected here if anywhere. Indeed the frequent degeneration of masses of cells in the testes (Child, '07*b*) seems to indicate very clearly that insufficiency of nutrition and failure to attain equilibrium exist, especially as this degeneration is much more frequent in some chains and proglottids than in others. Apparently some cells are forced so far from equilibrium that they can no longer exist. It has been noted (Child, '07*b*) that such degeneration of cells was not observed in stages from the beginning of the growth-period to the spermatid. It is not improbable that cells which once enter upon

the growth-period possess sufficient energy to obtain nutritive material, notwithstanding the demands of their rivals in earlier stages, but it is also possible that some of these cells are so far from a condition of equilibrium that they cannot go through the maturation mitoses following the growth-period. Such cells are probably those in which fragmentation occurs. Certainly the process of fragmentation in the spermatocytes (Child, '07*b*) presents no difficulties to such an interpretation. In it most of the nuclear substance disappears and a few small nuclei are formed from what might almost be regarded as the debris of the original nucleus. Apparently the old nucleus is no longer able to exist as a physiological system and small parts of it form new systems. The special form of fragmentation in these cases may be merely the result of special conditions which are not present in the primitive cells of earlier stages.

It is evident then that these cases of fragmentation in the spermatocytes can readily be interpreted in the same manner as the amitoses in other stages. Whether the resulting nuclei actually take part in the formation of spermatozoa is a question which cannot be decided at present.

The typical spermatocytic mitoses were observed more frequently in testes situated in the extreme lateral regions of the proglottids, fragmentation apparently being much more frequent in the middle regions (Child, '07*b*). This fact likewise is probably to be regarded as favoring the suggestions made above. As was pointed out, in the extreme lateral regions of the proglottid the testes are not as numerous as in the middle regions and the resorptive surfaces from which nutritive material may reach them are relatively greater than in the middle regions. It may well be that the cells in these testes attain more frequently than others a condition in which mitosis is possible.

As regards the frequency of amitosis in cleavage of the earliest stages the fact that nuclear division is always far in advance of cytoplasmic division, except in those blastomeres which divide mitotically (Child, '07*d*) seems to indicate the existence of a strong stimulus to nuclear division in the nuclei dividing amitotically. Apparently most of the nuclei are forced by some factor to divide much more rapidly than they acquire correlations with

the surrounding cytoplasm, and so do not attain a condition in which the cyclical processes characteristic of mitosis can occur. It is not possible to determine the nutritive conditions with any certainty at this stage. It is very evident in the later stages, however, that those regions of the egg where the nuclei are smallest and amitosis is most frequent are regions from which the yolk is absent (Child, '07*d*, Figs. 27, 29, 30, 31). In the yolk-bearing regions the nuclei divide much less frequently and more often mitotically than in other regions. These facts point in the same direction as those already cited with respect to other stages.

In the somatic structures of the proglottid, mitosis has never been observed except in the lateral regions of the proglottid and very rarely there. When mitosis has been observed in the development of the genital ducts it was usually at or near the periphery of the proliferating region, in one of the cells which was evidently less intimately involved in the proliferation than those nearer the middle. These facts are in line with the preceding. Moreover the somatic cells usually possess only a very small amount of undifferentiated cytoplasm and this may be a factor in determining the physiological condition of the nuclei and so the form of division.

It would appear then that the facts concerning occurrence and relative frequency of amitosis and mitosis in *Moniezia*, as well as in other forms, do not conflict with the suggestions made by way of interpretation. There can I think be little doubt that the two forms of division correspond to different physiological conditions in the nucleus. Judging from the visible phenomena, it also seems probable that mitosis is associated with cyclical, and amitosis with acyclical processes. The questions as to the availability of nutritive material and "equilibrium" are more obscure and complex, for they concern not merely the presence or absence of nutritive material outside of the cell, but, and probably chiefly, its availability within the cell and for the nucleus. Various factors, such as quantity and quality of cytoplasm, size, form, and condition of nucleus, etc., may conceivably play a part in determining the physiological conditions in the cell. Moreover, we know little regarding the nature of nuclear equilibrium. I have used the term merely with reference to a condition in which cyclical processes with periodical reversals occur.

Doubtless, also, there are characteristic differences in different species. In the life-history of *Moniezia*, for example, rapid and enormous growth is a characteristic feature. In such a form the stimulus to division and growth must be more powerful or else the nuclei must react more readily than in other species in which the life-history does not involve such extensive growth. Whichever the case, we may expect to find the acyclical nuclear phenomena more characteristic of the species with extensive or indefinite growth than of others, simply because in the former the amount of synthesis is much greater and relatively more rapid than in the latter. If amitosis is associated with these extreme assimilative conditions, as our hypothesis postulates, then its distribution and frequency will follow the same rule.

Similar differences may be expected in different organs and regions of the individual, and so far as our knowledge goes at present, they appear to exist. Amitosis seems to be more characteristic of rapidly growing or assimilating organs and regions than of other regions.

If these observations and suggestions are correct, we can no longer regard mitotic figures as the sole criterion of nuclear division in organisms. In many forms, such, for example, as *Planaria*, in mid-summer, when growth and nuclear division are very rapid and fission is occurring every few days, mitoses are rarely seen, but amitoses are very abundant. In various cases of form-regulation, which were formerly supposed to occur without cell-division because no mitoses were observed, amitosis is very frequent.

Furthermore, if amitosis may occur in the normal developmental cycle and if it is especially characteristic of regions of rapid growth, as the facts indicate, we cannot depend upon the distribution of mitotic figures in developing tissues as an indication of the rapidity of cell-division and growth in different regions. The regions where mitoses are most abundant may be the regions of slowest division instead of the only regions where division is occurring.

The bearing of these and other observations on certain cytological hypotheses is briefly discussed in another paper (Child, '07*c*) and requires no further consideration here. I hope in

future to continue these observations on other species and to offer further evidence for or against the hypothesis presented here, together with a more extended discussion of the observations of others.

April, 1907.

BIBLIOGRAPHY.

Child, C. M.

'07a Studies on the Relation between Amitosis and Mitosis. I. Development of the Ovaries and Oögenesis in Moniezia. Biol. Bull., Vol. XII., No. 2, 1907.

'07b Studies on the Relation between Amitosis and Mitosis. II. Development of the Testes and Spermatogenesis in Moniezia. Biol. Bull., Vol. XII., Nos. 3 and 4, 1907.

'07c Amitosis as a Factor in Normal and Regulatory Growth. Anat. Anz., Bd. XXX., Nos. 11 and 12, 1907.

'07d Studies on the Relation between Amitosis and Mitosis. III. Maturation, Fertilization, and Cleavage in Moniezia. Biol. Bull., Vol. XIII., No. 3, 1907.

Vom Rath, O.

'95 Ueber den feineren Bau der Drüsenzellen des Kopfes von *Anilocra mediterranea* Leach im Speciellen und die Amitosenfrage im Allgemeinen. Zeitschr. f. wiss. Zool., LX., 1895.

Ziegler, H. E.

'91 Die biologische Bedeutung der amitotischen (direkten) Kernteilung im Tierreich. Biol. Centralbl., Bd. XI., No. 12, 1891.

Ziegler, H. E., and O. Vom Rath.

'91 Die amitotische Kerntheilung bei den Arthropoden. Biol. Centralbl., Bd. XI., No. 24, 1891.

Explanation of Plate VIII.

Fig. 1. A very early stage of the developing genital ducts in the region lateral to the longitudinal nephridial canals. The width of the figure from right to left represents approximately the width of the region involved in proliferation and the axis of the duct corresponds with a line through the middle of the figure from top to bottom

Fig. 2. A somewhat later stage in the development of the genital ducts, from the same region and showing one case of mitosis. Plane of section as in Fig. 1.

Fig. 3. The developing vas deferens near its inner end. The width of the figure represents the width of the proliferating region. Plane of section as in Fig. 1.

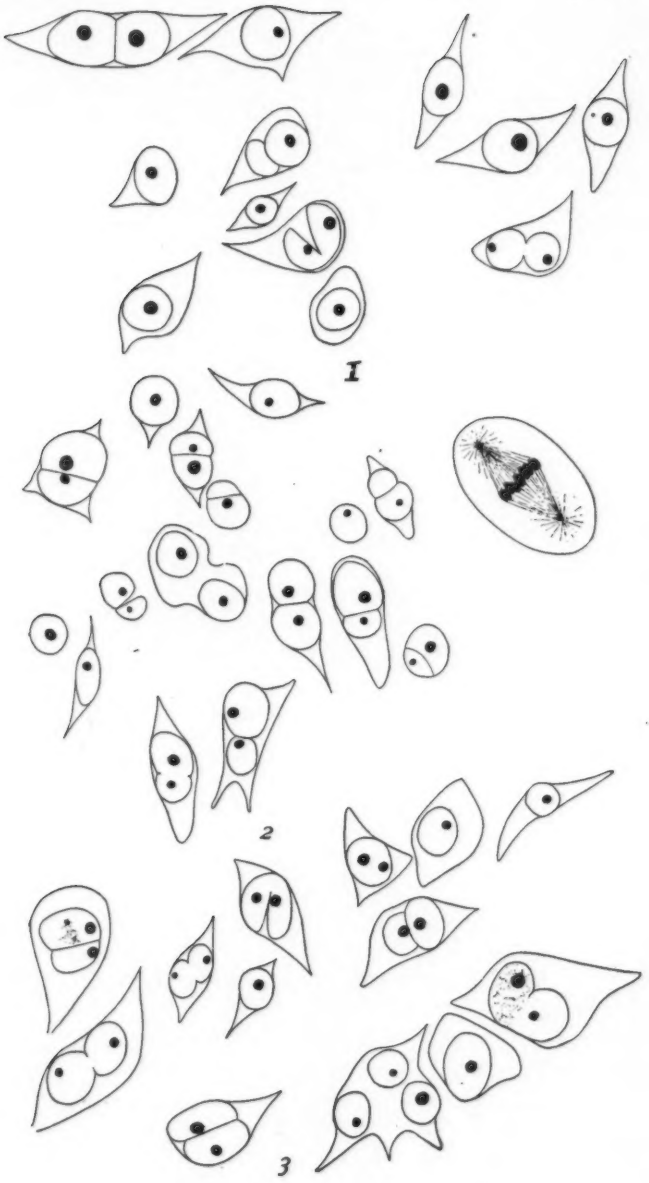
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PLATE IX.

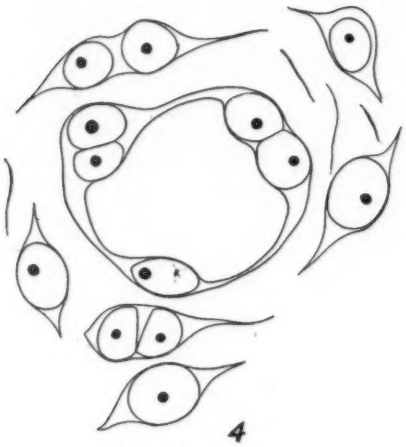
FIG. 4. Cross-section of the vas deferens at a somewhat later stage.

FIG. 5. Longitudinal section of a vas efferens.

FIG. 6. A portion of the wall of the developing oviduct, showing one case of mitosis.

FIG. 7. Probably a developing nephridial duct in cross-section.

FIG. 8. From the region of the lateral longitudinal nerve-cords in the "neck."



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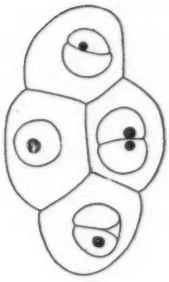

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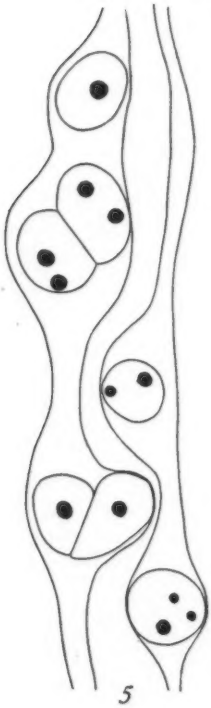

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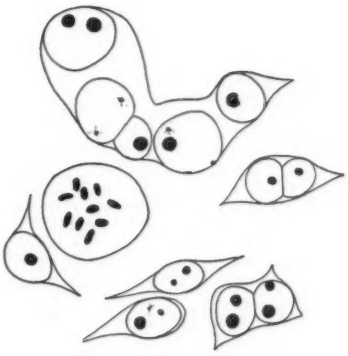

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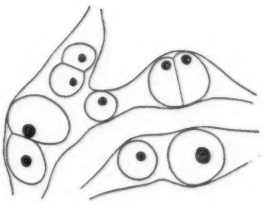

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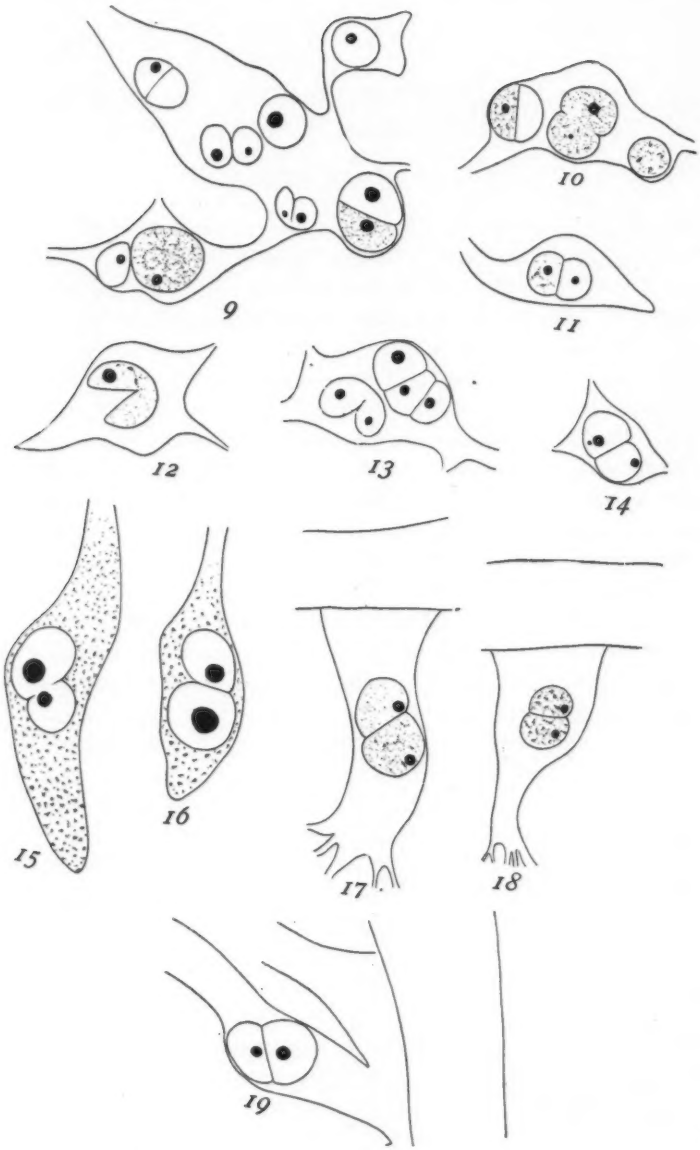
PLATE X.

FIG. 9. From the region of the lateral longitudinal nerve-cords in the "neck."
FIGS. 10–14. From the general parenchyma in neck-region.
FIGS. 15–16. Interproglottidal glands after differentiation has begun.
FIGS. 17, 18, 19. Cells of the subcuticular layer from the neck-region.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ON CERTAIN MODIFIED HAIRS PECULIAR TO THE ANTS OF ARID REGIONS.

WILLIAM MORTON WHEELER.

While studying the ants of the southwestern States I have been impressed with the series of unusually long hairs, or macrochætæ, which occur on the lower surface of the head and mandibles and on the anterior border of the clypeus in the workers and females of several species peculiar to the arid plains and deserts. Examination of the ants from similar localities in South America and from the still more arid regions of Africa, shows a prevalence of the same structures. There is also observable a tendency for these hairs to reach their greatest development in the species inhabiting the driest deserts. Although these structures have long been known to descriptive myrmecologists, no one, to my knowledge, has noticed the connection between their presence and a pronounced xerophily, or has endeavored to ascertain their function. These matters are of some interest, because the macrochætæ occur in several unrelated genera belonging to three of the five subfamilies of the Formicidæ, and therefore represent a striking example of convergent development. It is, furthermore, comparatively easy to account for the structures in question, as they are merely elongated and somewhat modified portions of the general hairy investiture of the ant's body.

Before treating of the function of these hairs and their occurrence in the various genera and subgenera, it will be advisable to give a general account of their arrangement. When most completely developed, they may be said to constitute four series, two paired and two unpaired as follows:

1. *Clypeal Macrochætæ.* — Although many ants have a fringe of long hairs or bristles on the anterior border of the clypeus, these are best developed in the desert species. They are curved downwards at their tips, are longest and most projecting on the free median portion of the clypeal border, and gradually grow shorter towards the lateral corners.

2. *Mandibular Macrochætæ.* — Both the dorsal and ventral surfaces of the mandibles in many ants are clothed with short, more or less projecting hairs, but the xerophilous species have in addition on each of the jaws, a ventral series of long and rather slender macrochætæ, which project downward and have their tips curved inward and sometimes upward. These hairs are longest on the bases and gradually become shorter towards the tips of the mandibles.

3. *Mental Macrochætæ.* — Some of the xerophilous species have a tuft of macrochætæ on the postero-median portion of the mentum, just in front of the anterior border of the gula. In one genus (*Ocymyrmex*) they extend backward and downward, in two others (*Myrmecocystus*, *Melophorus*) they project forward; in all cases their tips are turned forward or upward.

4. *Gular Macrochætæ.* — In most of the xerophilous species, these constitute the longest and most conspicuous hairs on the whole body. They are inserted, often in an arcuate series, on the posterior or lateral portions of the gula, are directed forward and downward and are often curved upward at their tips.[1]

As these various series of hairs or bristles together constitute a circumoral system, one is naturally inclined, on inquiring into their function, to suspect that there may be a connection between their development and some peculiarity in the feeding habits. It is true, to be sure, that these habits are apt to be highly specialized in desert ants. The natural and primitive food of ants consists of insects or the juices of plants, the latter collected either directly from the floral and extrafloral nectaries or indirectly after passing through the bodies of phytophthorous Homoptera (Aphididæ, Coccidæ, Membracidæ). But as insects and flowers are rare for long periods of the year in arid regions, many xero-

[1] The term *gula* is here used in the sense of Janet ("Recherches sur l'Anatomie de la Fourmi et Essai sur la Constitution Morphologique de la Tête de l'Insecte," Paris, G. Carré and C. Naud, 1900, 205 pp., 15 Pls.) as that portion of the cranial capsule which arises from the fused labial, maxillary and mandibular segments. It comprises the greater portion of the heavily chitinized antero-ventral integument of the head, and is divided into two parts by a median longitudinal suture, the external indication of the gular apodeme. In reality, as Janet has shown, the gular apodeme represents the whole labial and maxillary and the mesial portion of the mandibular segment of the cranium, so that the gula proper comprises not only the lateral, but also the larger portion of the mandibular segment.

philous ants have taken to harvesting and eating seeds. Others, like the honey ants (American species of *Myrmecocystus*, Australian *Camponotus* and *Melophorus*), still collect plant juices with avidity, but as such liquids are scarce or only temporarily abundant, they store them in the distensible crops of certain workers, which thus function as living bottles (repletes, or plerergates). Still other ants, like many *Myrmecocysti*, both in the Sahara and in the deserts of the southwestern States, have exaggerated the primitive entomophagous habit and have become very agile, predatory hunters.

As the circumoral macrochætæ occur in desert ants that have specialized in each of these three directions, it is difficult to detect any relation between the development of such structures and the character of the food. But inasmuch as the food of all ants really consists exclusively of liquids either imbibed directly or carefully expressed from moist solids, I was led at first to adopt the following hypothesis: When the mandibles are wholly or partially closed, the macrochætæ are seen to form a crate or lattice-work, enclosing a lenticular space on the ventral side of the head. A drop of liquid carefully introduced into this space by means of a fine pipette will fill it and hang securely suspended from the flat or concave gula, with the spherical surface supported by the hairs of the crate. This experiment led me to the opinion that the hairs might be used for one or both of two purposes: first, to retain regurgitated drops of liquid and prevent their falling to the earth while the ants are feeding their larvæ or one another; and second, to enable the ants to collect from the stones and desert plants drops of rain water and to carry these to their nests. The hairs would certainly seem to be admirably adapted to both of these liquid-saving functions. This hypothesis seemed to be confirmed by the following observation on our northern *Stenamma* (*Aphænogaster*) *fulvum*, published by Miss Fielde:[1] "I have observed that these ants, like the termites, are able to carry water for domestic purposes. They probably lap the water into the pouch above the lower lip and eject it at its destination. A hundred or two ants that I brought in and left in a heap of dry earth upon a Lubbock nest, during the ensuing night took

[1] "Further Study of an Ant," *Proc. Acad. Nat. Sci. Phila.*, 1901, pp. 521–544.

water from the surrounding moat, moistened a full pint of the earth, built therein a proper nest, and were busy depositing their larvæ in its recesses when I saw them on the following morning." Miss Fielde assures me that she has repeatedly observed this interesting occurrence, especially when the ants had larvæ or pupæ, to which contact with perfectly dry earth would, of course, soon be fatal. There is, therefore, nothing extravagant about the view that desert ants, living in very dry soil, might carry water in the macrochætal crate instead of in the crop or hypopharyngeal pocket.

In order to ascertain, if possible, the true state of affairs from the ants themselves, I requested Miss Augusta Rucker to send

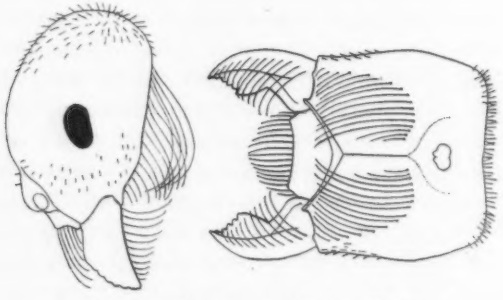

FIG. 1. *Pogonomyrmex barbatus* F. Sm.

me a number of living workers of the Texan harvester (*Pogonomyrmex barbatus* F. Smith var. *molefaciens* Buckley), a species with well-developed circumoral macrochætæ. The study of these insects in an artificial nest soon convinced me that my hypothesis, at least so far as this form is concerned, was erroneous. Though the ants were kept for several days without water and then given the liquid in small drops on the floor of their nest in imitation of the drops left by a shower on the stones and plants around the nests in their native environment, they were never seen to take it up into the macrochætal crate but simply lapped it up with their tongues. Protracted observation also proved that these ants never feed one another by regurgitation but that each worker partakes individually of the seeds, sugar, insects,

etc., brought into the nest. I had previously found that this species does not feed its larvæ by regurgitation but with pieces of seeds or insects.

Continuing my observation of the living harvesters I was soon led to what I believe to be the true function of the macrochætæ. Each of the fore legs, as in other Formicidæ, bears a well-developed strigil, or enlarged and pectinated spur (Fig. 2) which in the living insect is usually carried at an angle of about 70° with

FIG. 2. *Pogonomyrmex barbatus*, fore tarsus, mesial side.

the long axis of the tibia. The tibia is furnished at its tip on the mesial side with a dense brush of blunt hairs and the metatarsus is curved outward at the base, with a regular comb of fine bristles at the concavity and a series of coarser and blunter bristles along its whole mesial border. The pectinated strigil may be opposed to the pectinated and concave base of the metatarsus, and is especially adapted for cleaning the antennæ, which are drawn through the orifice between the two combs.

McCook[1] has described and figured (Pl. XIV., Fig. 64) the fore leg of *P. molefaciens*. I quote the following from his detailed account of the toilet habits (pp. 127–129): "The ants engaged in cleaning their own bodies have various modes of operating. The fore legs are drawn between the mandibles, and, so far as could be ascertained, also through or along the lips, and then are passed alternately back of the head, over and down the forehead and face, by a motion which closely resembles that of a cat when cleansing with its paw the corresponding part of her head. Sometimes but one side of the head is cleansed, in which case the foot used is drawn through the mandibles or across the teeth of one mandible after every two or three strokes upon the face.

[1] "The Natural History of the Agricultural Ant of Texas, a Monograph of the Habits, Architecture and Structure of *Pogonomyrmex barbatus*." Author's edition. *Acad. Nat. Sci. Phila.*, 1879.

These strokes are always made downward, following thus the direction of the hairs." . . . "Not only the fore pair, but also the other legs are passed — as above described — through the mouth. The second and third pairs are also and oftener cleansed by the fore legs, as follows: The ant throws herself over upon her side, draws up the middle and hind legs, which are interlocked at the tarsi, and then clasping them with one fore leg, presses the other downward along the other two. The fore legs alternate in this motion. When the legs on one side are cleansed, the ant reverses her position and repeats the process. When the antennæ are cleansed they appear to be taken between the curved spur at the extremity of the tibia and the tibia itself, as one would clasp an object between the base of the thumb and the hand, and are drawn along toward the tip of the flagellum evidently with one pressure." The cleansing of the abdomen or gaster is described as follows: "The hind legs are thrown backward and well extended, the middle pair nearly straight outward from the thorax, and less extended, so that the body is able to assume a nearly erect posture. The abdomen is then turned under the body and upward toward the head, which is at the same time bent over and downward. The body of the ant thus forms a letter C, or nearly a circle. The fore feet have meanwhile clasped the abdomen, and the work of brushing has begun. The strokes are directed upward toward the apex of the abdomen, and the foot passes around and beneath the under part, which is now toward the sternum, the apex is frequently licked by the tongue, and the feet are occasionally passed through the mouth (not simply between the mandibles), after which they are again applied as before."

This description is correct as far as it goes. The four cleaning reflexes, that of the antennæ, sides of the head, posterior legs and gaster, are distinctly differentiated and are so often repeated in the artificial nest that they can be readily studied under a lens of low magnification. These reflexes may be elicited with even greater frequency by powdering the ants with dry dust, chalk or plaster of Paris.

The cleaning of the antennæ, which is far and away the most frequent of these reflexes, is sometimes abbreviated, as when the

ant merely raises one of her fore feet, grasps the scape or funiculus of the antenna of the same side between the strigil and the curved base of the metatarsus, passes the opposed combs along the apical portion of the appendage and again places her foot on the ground. Very often, however, the foot is carried forward directly from the antenna, thrust between the partially opened mandibles and then drawn back across the teeth, along the lower surface of the mandible and between the maxilla and labium. The maxilla is furnished with a comb very much like that on the strigil.[1]

This motion, of course, removes much of the dirt that may have been collected from the surface of the antenna, since the strigil and the fore tarsus are drawn through the clypeal macrochætæ, across the mandibular teeth, along the mandibular macrochætæ and over the maxillary comb. The function of the gular macrochætæ is not, at first sight, so apparent. Occasionally, however, I have seen an ant, while thrusting her fore foot forward, enclose between the strigil and metatarsus one or more of the long gular hairs, and draw them through the combs or along the notch between the insertions of the strigil and metatarsus. This observation, coupled with the fact that these hairs are very long, slender and directed forward, makes it highly probable that they are used for cleaning the strigil, much as we would use threads in cleaning a comb. In ants like the Old World species of *Myrmecocystus* (Figs. 8 and 9) which possess long mental but very poorly developed gular macrochætæ, the former probably answer the same purpose. The advantage to xerophilous ants of possessing a number of macrochætal brushes in addition to the strigils is obvious when we stop to consider that these insects live in dry soil, which, during long seasons of the year, is in a very friable and dusty condition. Both while traversing the surface in search of food and while carrying on their excavations, these ants are liable to become coated with dust or sand. Under such conditions the strigil must often become clogged with earthy particles or sand grains, and an apparatus for cleaning it, such as the gular and mental macro-

[1] See Janet's figures of the mouthparts of *Myrmica rubra* in his paper entitled: "Observations sur les Fourmis," Limoges, 1904, pp. 18 and 19.

chætæ, and hairs like those on the clypeus and mandibles for brushing the fore tarsi, must be of considerable utility. Ants living in sandy deserts often have the gular hairs unusually long, and well developed, probably because sand particles, with their sharp edges, are more injurious to the delicate combs of the strigil and metatarsus and interfere more with their normal movements of occlusion than do particles of soil. If, as I believe, the circumoral macrochætæ have the function here assigned to them, they may be designated as *ammochætæ* to distinguish them from the long hairs or bristles on other portions of the ant's body.

The absence of ammochætæ in several desert ants, such as the species of *Monomorium* and *Pheidole*, is probably to be explained by the very small size of the workers in both of these genera, and the fact that the soldiers of *Pheidole* do not excavate and rarely leave the galleries of the nest. For the same reason the ammochætæ are usually absent or feebly developed in the males of xerophilous species. That the workers and females of several other desert ants belonging to the genera *Solenopsis*, *Cremastogaster*, *Camponotus*, etc., should lack these structures is no more surprising than that many desert plants have failed to acquire the peculiar adaptive characters of the Cactaceæ. We may now consider briefly the occurrence of the ammochætæ in the various genera of xerophilous ants.

Subfamily Myrmicinæ.

Pogonomyrmex Mayr. — This genus embraces a number of species peculiar to North and South America and assignable to three subgenera, *Janetia* Forel, *Ephebomyrmex* Wheeler and *Pogonomyrmex* s. str. The single known species of *Janetia* (*J. mayri* Forel) occurs in Colombia. *Ephebomyrmex* comprises some four species, *E. nägelii* Forel of Brazil, *schmitti* Forel of Haiti, *imberbiculus* Wheeler of Texas and *townsendi* sp. nov. of Chihuahua. None of the species of these two subgenera has ammochætæ, and it should be noted that *J. mayri* and the first two species of *Ephebomyrmex* occur in comparatively humid regions, and that *E. imberbiculus* and *townsendi* though xerophilous, are not true desert ants. *Pogonomyrmex* s. str. comprises some twenty species, spread over portions of the high arid plains

and deserts of two continents, from Montana to Patagonia, with a possible interruption in tropical Central and South America. One of the species, *P. badius* Fabr., is found in the dry, sandy regions of Georgia and Florida. All of the members of this subgenus have very well developed clypeal, mandibular and gular ammochætæ, as shown in Fig. 1, representing the head of the Mexican and Texan *P. barbatus* F. Smith, the type of the subgenus. In certain species, like *P. californicus* Buckley, which is almost exclusively confined to sandy spots in the deserts of the southwest, the gular hairs are even longer and more prominent. The species of *Pogonomyrmex* s. str. and *Ephebomyrmex* are harvesters and subsist very largely on stored seeds. According to Forel *Janetia mayri* is entomophagous. There is reason to suppose that the genus *Pogonomyrmex* represents a granivorous American offshoot of the subboreal genus *Myrmica* or of some similar but now extinct group.

Ocymyrmex Emery. — This genus was erected by Emery for four species (*barbiger*, *nitidulus*, *robecchi* and *weitzekeri*) which he described from Somaliland, Basutoland and the Cape of Good Hope. It is probable that all of these species, which resemble

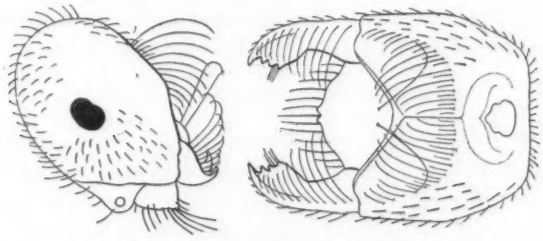

FIG. 3. *Ocymyrmex weitzekeri* Em.

Pogonomyrmex, live in the dry plains and feed on seeds. I have examined a few workers of *O. barbiger* and *weitzekeri* and find that they agree in having well-developed clypeal, mandibular and gular ammochætæ, and also a pair of long hairs on the mentum. These hairs, as shown in Fig. 3, extend backward, diverging from their insertions, and have their tips abruptly bent forward. The gular hairs are alternately long and short, and

the longer ones are less reclinate and shorter than in *Pogonomyrmex*. They are slightly curved upward at their tips. The mandibular ammochætæ are unusually long and curved.

Cratomyrmex Emery. — This genus is based on a single species, *C. regalis*, which Emery described from two large female specimens (19 mm. long), taken at Benue in western Africa.[1] They resemble *Pogonomyrmex* in having long hairs on the lower surface of the head, but these hairs are said to be shorter and less regularly arranged than in *P. barbatus*.

Stenamma Mayr. — This extensive genus may be divided into six subgenera: *Stenamma* s. str., *Aphænogaster*, *Ischnomyrmex*, *Messor*, *Goniomma* and *Oxyopomyrmex*. The species of *Stenamma* s. str., are moisture-loving ants living in small colonies in the woods of the north temperate zone. The much more numerous species of *Aphænogaster* are widely distributed and occur in a great variety of environments, some even living in dry deserts, but none of them seems to have developed ammochætæ, although the lower surface of the head, like the body in general, is usually provided with coarse erect hairs. From species of this or some very similar group the remaining subgenera, *Ischnomyrmex*, *Messor*, *Goniomma* and *Oxyopomyrmex*, seem to have been derived. All of these comprise xerophilous or deserticolous species with ammochætæ and seed-eating propensities.

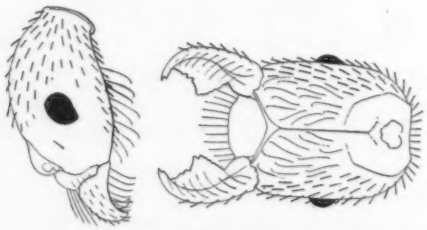

FIG. 4. *Ischnomyrmex albisetosus* Mayr.

Ischnomyrmex Mayr. — Our two North American species of this subgenus, *I. cockerelli* André and *albisetosus* Mayr are closely related and both inhabit the driest deserts of western Texas, New

[1] Voyage de M. Ch. Alluand dans le territoire d'Assinie (Afrique Occidentale), Formicidæ. *Ann. Soc. Ent. France*, LX., 1891, pp. 553–574, Pl. XV.

Mexico, Arizona and northern Mexico. We should therefore expect them to have well-developed ammochætæ. These are, in fact, present in both species, but, as shown in Fig. 4, the gular hairs are diffuse and not arranged in an arcuate series. They are, however, longer and more slender than the blunt, white hairs covering the remainder of the body, and are distinctly hooked or curved upward at their tips. This peculiar modification of the gular hairs is not seen in the other members of the subgenus inhabiting more humid regions, like *I. araneoides* Emery of Central America, *swammerdami* Forel of Madagascar, and *longipes* F. Smith of Burmah and Sumatra. In pilosity these resemble our northern species of *Aphænogaster*.

Messor Forel. — This subgenus of harvesting ants is represented by a number of species, subspecies and varieties in Africa,

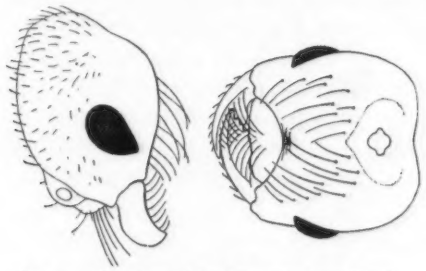

FIG. 5. *Goniomma blanci*, v. *tuneticum* Forel.

especially in the Sahara, in southern and central Europe and Asia and by five species in Arizona, Nevada, California and northwestern Mexico. In both hemispheres the xerophilous species have well-developed ammochætæ, whereas these structures are small or lacking in the moisture-loving forms. To the latter group belongs the widely distributed *M. barbarus* L. which even in Africa, according to Forel "vit dans les lieux moins secs; fait souvent des nids maçonnés dans la terre, dans les prairies,"[1] and Lameere[2] says: "Je n'ai rencontré le type de cette moissonneuse que dans les parties cultivées des oasis; dans le désert cail-

[1] See Emery, "Revision Critique des Fourmis de la Tunisie, in Exploration Scientifique de la Tunisie," Paris, 1891, p. 10.

[2] "Note sur les Moeurs des Fourmis du Sahara," *Ann. Soc. Ent. Belg.*, XLVI., 1903, pp. 160–169.

louteux, alluvial, j'ai toujours trouvé la race *ægyptiacus* Emery, et dans le désert rocailleux la race *striaticeps* André." Now the race or subspecies *ægyptiacus* has well-developed ammochætæ, and in *striaticeps* these bristles are also present, though more feebly developed. *M. arenarius* Fabr., and *caviceps* Forel, two species peculiar to the dry sandy portions of the Sahara, and apparently also *M. bugnioni* Forel of the same region, have highly developed ammochætæ, but these are absent in the European *M. structor*, and in *lobicornis*, a form discovered by Forel in the oasis at Biskra.[1]

Among our American species, *M. pergandei* Mayr and *julianus* Pergande have well-developed ammochætæ, whereas *andrei*

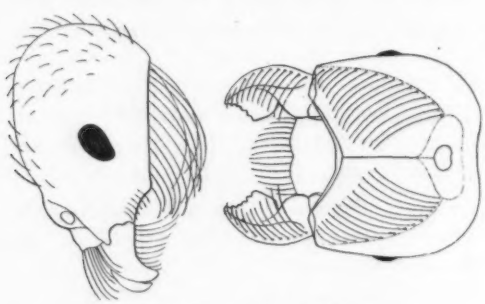

FIG. 6. *Messor pergandei* Mayr.

Mayr, *carbonarius* Pergande and *stoddardi* Emery have only the usual hairs on the lower surface of the head. The habits of *carbonarius*, *julianus* and *stoddardi* are unknown but *pergandei*, as I can assert from my own observations, lives only in the driest portions of the deserts of Arizona and California, and *andrei* and *stoddardi* are cited only from more humid portions of the latter state. The head of *pergandei*, which is represented in Fig. 6, shows a fine development of the clypeal, mandibular and gular bristles. The gular series form an arc on each side bounding a distinctly concave median region separated from the convex lower portions of the cheek by a distinct ridge (represented in the figure by a dotted line along the insertions of the bristles).

[1] "Les Formicides de la Province d'Oran (Algerie)," *Bull. Soc. Vaud. Sci. Nat.* XXX., no. 114, 1894, pp. 31–33.

Goniomma Ern. André. — Of the few forms that have been assigned to this Mediterranean genus, I have examined only *G. blanci* André var. *tuneticum* Forel from the Sahara. The head of the worker, represented in Fig. 5, shows well-developed clypeal and mandibular ammochætæ. On the gula, however, which has no distinct median suture, the bristles are diffuse and only moderately developed, and not arranged in an arc. This ant is probably granivorous like *G. hispanicum*, whose habits have been studied by Forel.

Oxyopomyrmex Ern. André. — This group, like the preceding, comprises a few small Mediterranean species. I have been able to examine a number of workers and females of *O. santschii* Forel kindly sent me by Dr. F. Santschi of Kairouan, Tunis. The ammochætæ are similar to those of *Goniomma tuneticum*. The gular bristles are diffuse and rather short, though conspicuously longer than the hairs on other parts of the body. Santschi has recently shown that this ant garners seeds.[1]

Both *Goniomma* and *Oxyopomyrmex* seem to represent depauperate offshoots of the grain-storing portion of the genus *Stenamma* (*Messor*). The depauperate character is apparent in the small size of the insects and their colonies and in the vestigial condition of the gular ammochætæ.

Holcomyrmex Mayr. — This genus of harvesting ants, apparently confined to southern Asia and northern Africa, was at first regarded by Emery[2] as being hardly distinct from *Stenamma*, but somewhat later[3] he says: "Dans ma clef analytique des genres des formicides j'ai exprimé des doutes sur la validité des caractères qui séparent le genre *Holcomyrmex* de *Stenamma*. Toutefois l'aiguillon est bien développé chez *Holcomyrmex*, faible ou rudimentaire chez tous les sous-genres de

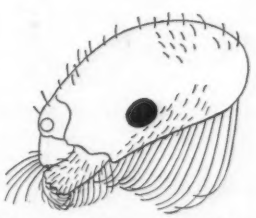

FIG. 7. *Holcomyrmex chobauti* Emery.

[1] See Forel, "Miscellanea Myrmécologiques, *Rev. Suisse Zool.*, T. XII., 1904.

[2] Clef Analytique des Genres de la Famille des Formicides, pour la Determination des Neutres," *Ann. Soc. Ent. Belg.*, T. XL., 1896, p. 185, *nota*.

[3] "Description d'une Fourmi Nouvelle d'Algérie," *Bull. Soc. Ent. France*, 1896, p. 419.

Stenamma. Ce charactère qui m' avait échappé à son importance me porte à croire que les analogies frappantes entre les deux genres sont dues à une adaptation convergente. *Holcomyrmex* est la modification granivore de *Monomorium*, comme *Messor* est celle de *Stenamma* (*Aphænogaster*)." There are no ammochætæ in the Asiatic species of *Holcomyrmex* (*criniceps* Mayr and *scabriceps* Mayr of India), though the lower surface of the head is abundantly pilose like the remainder of the body. These species evidently inhabit rather humid regions. In the species from the dry Sahara (*H. chobauti* Emery and *faf* Forel) the ammochætæ are beautifully developed. In *chobauti* (Fig. 7) the gular bristles are very long and inserted in an arcuate series, and the head is very flat, with concave gular surface. Forel describes *H. faf* as having long red hairs on the lower surface of the head. In *H. lameerei* Forel, which also inhabits the Sahara, the gular hairs are shorter, straighter and diffuse, but nevertheless abundant.

Subfamily Camponotinæ.

Myrmecocystus Wesmæl. — This genus includes the only prominent and characteristic Camponotine ants common to the arid regions of both hemispheres. The Old World spe-

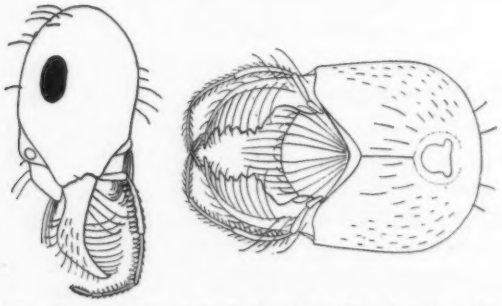

Fig. 8. *Myrmecocystus bicolor* F. (= *M. viaticus desertorum* Forel).

cies, ranging from the plains of central Asia through central and southern Europe and north Africa to Spain and Morocco, are all very agile, entomophagous ants, which run rapidly over the dry, sunny soil in pursuit of their food. In nearly all of these

palearctic forms the clypeal and mandibular ammochætæ are well developed. The gular bristles, however, are vestigial, their function being usurped by a fan-shaped tuft of long recurved hairs on the mentum. It is a significant fact that the hairs of this tuft are rather short in *M. cursor* Fonsc., a species which, according to Emery does not occur in Africa.[1] It belonged originally to the central Asiatic fauna, but just after the glacial period migrated into central and southern Europe. In the north African forms (*M. albicans* Roger, *viaticus* Fabr., *bicolor* Fabr. (= *viaticus desertorum* Forel), *bicolor megalocola* Foerster) the ammochætæ, especially those on the mentum, are longer (Fig. 8). In *M. bombycinus* Roger, a typical Saharan species, the bristles reach their highest development (Fig. 9), and there are also fringes of long curved hairs on the third joint of the greatly elongated maxillary palpi so characteristic of the ants of this genus.

There seem to be only two American species of *Myrmecocystus*,

FIG. 9. *Myrmecocystus bombycinus* Roger.

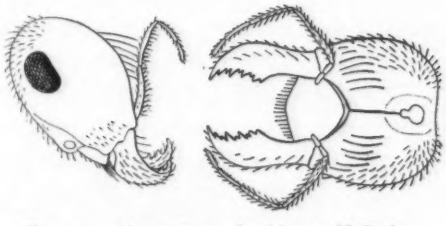

FIG. 10. *Myrmecocystus hortideorum* McCook.

mexicanus Wesm. and *melliger* Forel, but each of these has a number of subspecies and varieties, still in part undescribed and all confined to the arid plains and deserts of the southwestern States and northern Mexico. Some of the forms of each of the species collect the secretions of plants and the ejecta of aphids and store the liquids thus obtained in the replete workers ("honey ants"). Other subspecies and varieties do not seem to have this

[1] "Rassegna Critica delle Specie Paleartiche del Genere Myrmecocystus," *Mem. R. Accad. Sci. Lt. Bologna*, 1906, pp. 1–17, 35 figs.

habit but live on insect food. The clypeal and mandibular ammochætæ are smaller than in the palearctic species. There are no hairs on the mentum, but the gular bristles are long, arcuately inserted, rather stiff, directed downward and but little curved. The development of these, as well as that of the clypeal and mandibular ammochætæ varies directly as the aridity of

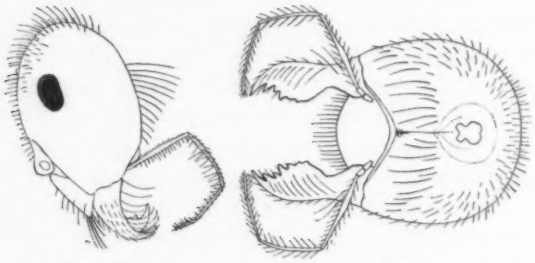

FIG. 11. *Myrmecocystus melliger orbiceps* sp. nov.

the region inhabited by the ants. Thus *M. mexicanus* var. *hortideorum* McCook (Fig. 10) which lives on the high plains in comparatively humid regions, has very poorly developed clypeal and mandibular bristles and the gular ammochætæ are rather short. In *M. melliger orbiceps* subsp. nov. (Fig. 11) which inhabits the drier regions of central and western Texas, all of the bristles are

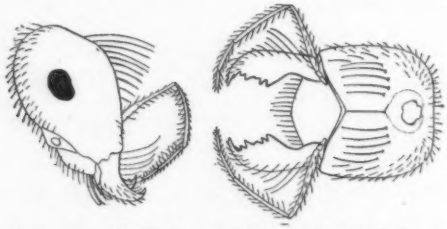

FIG. 12. *Myrmecocystus melliger semirufus* Emery.

longer. Finally *M. melliger semirufus* Emery (Fig. 12), a small form peculiar to the sandy spots in the dry deserts of Arizona and southern California, surpasses all the other American forms in the development of the ammochætæ.

The uniform presence of mental ammochætæ and the larger size of the males in the Old World forms of *Myrmecocystus* would

seem to indicate that these are at least subgenerically distinct from the American forms. It may be advisable, therefore, to reinstate for the palearctic members of the genus the name *Cataglyphis* published by Foerster in 1850. The name *Myrmecocystus*, established in 1838 by Wesmael for *mexicanus*, would then comprise the American species.

Melophorus Lubbock. — This interesting genus embraces three subgenera: *Melophorus* s. str., *Prolasius* Forel and *Lasiophanes* Emery, all peculiar to the southern hemisphere. Many of the species of *Melophorus* proper resemble *Myrmecocystus*. In *M. bagoti* Lubbock and *M. wheeleri* Forel, which inhabit the most arid portions of Australia and have dimorphic workers (*M. bagoti*, at least, being a honey ant, as Lubbock has shown!), the ammochætæ are highly developed and all the series are present, even to the tuft on the mentum. The gular series are longer and more abundant than in the American species of *Myrmecocystus*. These hairs are less developed in *M. iridescens* Emery, *æneovirens* Lowne, *curtus* Forel, *hirtus* Forel, and *ludius* Forel, as I have found from an examination of specimens of all of these forms in Professor Forel's collection. In *M. nitidissimus* André and *formicoides* Forel all the series of ammochætæ are inconspicuous, so that these forms may be taken to represent a transition to the *Lasiophanes* species of Chile and *Prolasius advena* F. Smith of New Zealand, which are not deserticolous and have no prominent hairs on the lower surface of the head and mandibles.

Subfamily Dolichoderinæ.

Dorymyrmex Roger. — This peculiarly American genus is the only one of the Dolichoderine subfamily in which I have found ammochætæ. A single species, *D. pyramicus* Roger, with at least three varieties (*niger* Pergande, *flavus* McCook and *bicolor* Wheeler) is widely distributed through the subtropical and tropical portions of both continents. It lives exclusively in dry soil or sand, preferably in the latter. More numerous species occur in the dry regions of Argentina, Patagonia and Chile, which probably represent the original home of the genus. Several of these species (*D. planidens* Mayr, *mucronatus* Emery, *tener* Mayr and *baeri* André) have well-developed ammochætæ especially on

the clypeus and gula. I find that the conditions shown in Fig. 13, taken from one of Emery's recent papers,[1] obtain in all of these species. The gular macrochætæ are arranged in an arc on each side and the insertion of each bristle is marked by a black

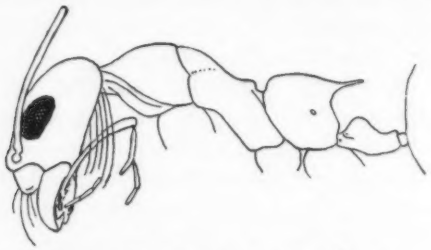

FIG. 13. *Dorymyrmex mucronatus* Emery.

dot. In our North American forms of *D. pyramicus* (Fig. 14), the ammochætæ are reduced to a very prominent series on the clypeus and a few short bristles, inserted in a short irregular arc on

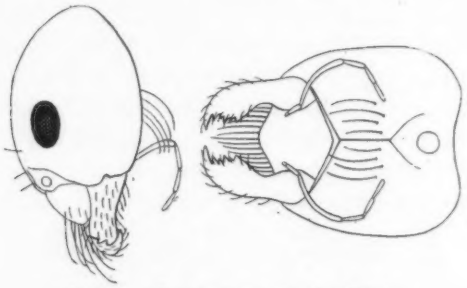

FIG. 14. *Dorymyrmex pyramicus* Roger.

the middle of the gula. The latter, which are all the more conspicuous because the remainder of the body is nearly destitute of hairs, nevertheless represent what may be regarded as a degenerate condition.

[1] "Studi sulle Formiche della Fauna Neotropica," *Bull. Soc. Ent. Ital.*, XXXVII., 1905, Fig. 34.

CAN SEA WATER MAINTAIN THE BEAT OF THE HEART OF FRESH WATER ANIMALS?

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In 1900 Loeb[1] introduced the idea of physiologically balanced solutions, *i. e.*, "salt solutions which contain such ions in such proportions as to annihilate completely the poisonous effect which each constituent would have if it were alone in solution." On this basis blood, sea water and Ringer's solution are examples of balanced solutions. Loeb[2] was led to this idea by the observation that young *Fundulus*, which live in sea water, can also live in distilled water, while they die rapidly in a pure solution of sodium chloride (or any other salt) of the same osmotic pressure as sea water. Now we know that the life of the heart is sustained in blood and in Ringer's solution; the question arises is it equally well sustained in sea water that has been made isotonic with the blood. We have already a few facts in this regard. Rogers[3] found normal sea water to be an excellent sustaining fluid for the heart of the marine crab, and Garrey,[4] says "the mammalian muscle lives longer in isotonic sea water than in any other inorganic solution tested." Osterhout[5] experimenting with *Vaucheria sessilis*, a green alga common in running water, found it would grow well and live indefinitely in sea water with a concentration approximating 3/32 *M.* According to Van't Hoff the composition of sea water is as follows: 100 mols. NaCl; 2.2

[1] Loeb, J., "On the Artificial Production of Normal Larvæ from the Unfertillized Eggs of the Sea Urchin (Arbacia)," *Am. Jour. Phys.*, Vol. 3, 1900, p. 434. Also "Studies in General Physiology," p. 590.

[2] Loeb, J., "On Ion-proteid Compounds and Their Rôle in the Mechanics of Life Phenomena," *Am. Jour. Phys.*, Vol. 3, 1900, p. 327.

[3] Rogers, C. G., "The Effects of Various Salts upon the Invertebrate Heart," *Jour. Exper. Zoöl.*, Vol. 2, 1905, p. 237.

[4] Garrey, Walter E., "Twitchings of Skeletal Muscles Produced by Salt Solutions, etc.," *Am. Jour. Phys.*, Vol. 13, 1905, p. 186.

[5] Osterhout, W. J. V., "Extreme Toxicity of Sodium Chloride and its Prevention by Other Salts," *Jour. Biol. Chem.*, V ol. 1, 1905–6, p. 363.

mols. KCl; 2 mols. $CaCl_2$; 7.8 mols. $MgCl_2$; 3.8 mols. $MgSO_4$; reaction alkaline. Sea water thus differs from Ringer's solution in containing magnesium, while it differs from blood only in the excessive amount of magnesium present. This being the case it was determined to try its effect on the heart of the fresh water turtle.

The technique employed was essentially that used by physiologists in experiments of this nature. Strips of the ventricle were prepared in the usual way and attached by means of platinum hooks to a light aluminium lever magnifying about nine times. In order to have controls the strip was sometimes divided longitudinally, and sometimes transversely, as Martin[1] has shown there is no difference in the action of the strips (except the amplitude), whether prepared one way or the other. By dividing transversely strips are obtained containing fibers of both the anterior and posterior wall of the ventricle, which appeals to one as being advantageous for comparison. The lever was not weighted except when the whole heart was used, in which case a one gram weight was added just far enough from the fulcrum to about balance the weight of the heart. The lower end of the strip was attached to a glass rod bent at right angles, which served as a fixed point. Cylinders containing about thirty-five cubic centimeters of solution were used to immerse the strips. In the case of the sinus and auricles, they were separated completely from the ventricle by an incision in the auriculo-ventricular groove, and suspended in the same way as were the ventricular strips. The sea water used was taken from the Pacific Ocean at a point about a mile below the Cliff House, San Francisco. It had a freezing depression of 1.85. It was made isotonic by dilution with water distilled in glass, and its concentration determined from time to time by the freezing point. Twenty-eight c.c. of sea water in 100 gave the same depression as the Ringer's solution used for controls, which was made up according to the following formula: 100 mols. NaCl; 2 mols. KCl; 2 mols. $CaCl_2$; trace of $NaHCO_3$; all of $m/8$ concentration. Unless otherwise stated when "Ringer" is referred to, this solution is meant.

[1] Martin, E. G., "An Experimental Study of the Rhythmical Activity of Isolated Strips of Heart Muscle," *Am. Jour. Phys.*, Vol. 11, 1904, p. 103.

The turtles used were mostly those common to this locality (California), but a few were from Illinois — the *Emys Meleagris.*

It may be as well to begin by stating that the turtle's heart will remain alive as long in dilute sea water as in "Ringer." By "alive" is meant that condition of the heart in which, though quiescent it still responds to a stimulus by a contraction. Usually an induction current was used to ascertain if the heart would still contract. Individual hearts vary within such wide limits that in experiments on the whole heart controls were considered of such doubtful value that they were seldom used. The temperature also is a factor, for in warm weather bacterial decomposition soon sets in. In cool weather records of the ventricle have been obtained extending over one hnndred hours; but most of this work was done at higher temperatures and the hearts would not last as long either in sea water or in "Ringer." The auricles have given an uninterrupted series of contractions lasting eighteen hours or more, in cool weather.

When a strip of ventricle from a turtle's heart is immersed in isotonic sea water, it remains perfectly quiet indefinitely. Occasionally it will give a single spontaneous contraction, but it has been known to remain for twenty-four hours without giving a single beat. The shortest time in any of my experiments that it remained quiet was three hours and three quarters, when it gave one contraction, followed by two more eighteen hours later. Control strips in "Ringer" would invariably give single contractions or groups of contractions, separated by periods of quiescence, and were always more active than the corresponding strips in sea water. If, however, the strips were first treated with pure NaCl in $m/8$ concentration until "sodium chloride arrest" was induced, the difference was more marked. The strip transferred to "Ringer" usually recovered and gave a good series of contractions; while that transferred to sea water would revive for a few minutes and then become quiet, giving contractions of a very slow rate, or even none at all. That it was still capable of contraction was demonstrated by its response to stimulation with the induced current.

In striking contrast to the apical strips was the behavior of the auricles, always taken from the same heart for comparison. As

is well known when they are suspended the auricles contract spontaneously. When placed in dilute sea water the rate was always increased by several beats per minute, and they gave an uninterrupted series of contractions gradually diminishing in rate and amplitude, lasting from seven to twenty-eight hours. Usually when longer than twelve to eighteen hours, towards the last the contractions would be irregular, with pauses between groups. Control auricles in "Ringer" showed practically no difference. They too exhibited a slight increase in rate and gave the same characteristic series of contractions — the average being about fourteen hours in both "Ringer" and sea water, with the longest and the shortest records to the credit of the sea water. The following description of a few experiments will supply some details.

Exp. Jan. 28/'07; temperature 17° C. Strip *A* was placed in Ringer's solution. During the first four hours no contractions occurred. During the next four hours there were four series of rapid contractions, each series lasting from two to five minutes, with one half to one hour between. In ten hours from beginning the experiment the strip failed to respond to the induced current. Strip *B* was placed in dilute sea water. During ten hours it did not give a single contraction. At the end of this time it still responded feebly to the induced current. The auricle of this heart was suspended in dilute sea water and gave a fine series of contractions lasting about three hours. Before immersion the rate was sixteen per minute; after immersion it was eighteen, becoming slower toward the end of the three hours. This was the shortest series of contractions obtained. For some unaccountable reason both the ventricle and auricles gave out in a much shorter time than usual.

Exp. Feb. 1/'07; temperature 20° C. Two strips of the ventricle were prepared in the usual way. Strip *A* was put in Ringer's solution, and after a latent period of twenty-five minutes, gave a single vigorous contraction. It then remained quiet for twenty minutes, when it again began to contract, and gave a series of contractions lasting about ten hours, the rate varying from one to eight per minute. Twenty-two hours after beginning the experiment it was stimulated with the induced current, but gave no response. Strip *B* in dilute sea water remained perfectly

quiet for eleven hours. It was then stimulated with the induced current, and gave five vigorous contractions of maximum amplitude after which it was again quiescent. Twenty-two hours after beginning the experiment, it was again stimulated and gave a fairly good contraction of about one third the maximum in height. The solution was turbid indicating bacteria, and two hours later it failed to respond to stimulation. The auricle of this heart was placed in "Ringer" and gave a fine series of contractions lasting eleven and one half hours, with an initial rate of twenty, which was increased to twenty-four after immersion in "Ringer."

Exp. Feb. 4/'07; temperature 18° C. Two strips of the turtle's heart were prepared as usual. Both strips were put in *m*/8 NaCl in order to bring them to "sodium chloride arrest." Strip *A* began contracting after a latent period of fifteen minutes; its rate, five per minute, increasing to fifteen with gradually diminishing amplitude. At the end of two hours and fifteen minutes it ceased contracting. After remaining quiet for fifteen minutes it was transferred to "Ringer." It began at once to recover, but the contractions were small in amplitude, accompanied by a strong rise of tone. The rate was nine, increasing to fifteen per minute. Nine and one half hours from beginning the experiment, it responded to electrical stimulation by a very feeble twitch. Strip *B* began contracting after a latent period of fifteen minutes. Its rate was four per minute increasing to twelve. It came to "sodium chloride arrest" at the same time as did strip *A*, and was transferred to dilute sea water. It began contracting at once with a rate of nine per minute. After about fifteen minutes it ceased suddenly for five minutes and then began centracting feebly and in groups. It then settled down to a slow rate of one or two per minute, but with increased amplitude, and continued this for four and one half hours. It was stimulated with the induced current at the same time as was strip *A*, and gave a single contraction that was more vigorous than strip *A*, although it was still feeble. The auricle was contracting in the air at a rate of twenty per minute. After it was put in sea water the rate was twenty-four. It gave an uninterrupted series of contractions lasting about nine hours. After a long rest it recovered a little and gave some weak contractions. The duration of its irritabil-

ity was about nineteen hours, nine of which were spent in vigorous activity.

These three experiments were selected because they came in a series, and it so happens that it includes two of those that are the least typical of any. Exp. Jan. 28/'07 as has been said, lived for a much shorter time than usual, while in exp. Feb. 4/'07 the turtle was blind and sluggish; when the plastron was removed the heart was not contracting, nor did the auricles contract until they were suspended.

Exp. Feb. 8/'07; temperature 17–21° C. Two strips of the turtle's ventricle were prepared and exhausted in NaCl. Strip *A* was then transferred to dilute sea water. At the end of six minutes it began contracting vigorously with a much greater amplitude than in NaCl; at first with a rate of six per minute, but soon dropping to one in two or three minutes. In about fifteen minutes it ceased abruptly and remained perfectly quiet for about three quarters of an hour. It was then transferred to "Ringer," and in seven minutes began giving maximum contractions with a somewhat irregular rhythm; there would be a group of six to eight per minute, then a pause for half a minute and then another group. When it had been contracting in this way for one and one half hours it was again transferred to sea water. It was then registering on a twelve hour drum and the rate was not taken; but in fifteen minutes it again ceased abruptly and remained perfectly quiet for between five and six hours. Again transferred to "Ringer," it remained quiet for half an hour and then began to contract vigorously, but with gradually diminishing amplitude, and during the night it ceased. Next morning it failed to respond to stimulation. Strip *B* was used as a control. After NaCl exhaustion it was placed in "Ringer" instead of sea water, and recovered at once with a rate of sixteen gradually dropping to twelve per minute. The subsequent course of this strip was similar to strip *A*; when *A* was in "Ringer," *B* was in sea water and *vice versa*. When *A* was active in "Ringer," *B* was quiescent in sea water. The record of the auricles was lost after seven hours.

Having ascertained the influence of dilute sea water upon the different parts of the heart separately, the whole heart was sus-

pended by passing a hook through the apex below and one through the auricle above. In this way one can obtain a record of both the ventricular and the auricular contractions in their relations one to the other. The results of several experiments show that sea water favors the relaxation of the ventricle, while it has either no effect or a slightly stimulating one upon the auricles. The tracings show a more orderly sequence of auricular systole followed by ventricular systole, than in "Ringer," where the auricular systole has a tendency to fuse with the ventricular, owing possibly to incomplete relaxation of the ventricle. The question is being studied further in connection with the effects of magnesium on the heart; and as this paper is in the nature of a preliminary communication, the discussion of it will be left to a future date.

As the only practical difference between sea water and "Ringer" is the magnesium present in the former, it seems more than likely that the latter salt is responsible for the difference in the effect of these two solutions. As in the case of the marine crab, so in the turtle's heart, sea water rendered isotonic with the blood seems to be an excellent sustaining fluid; in other words sea water isotonic with the blood is a "physiologically balanced solution" for the turtle's heart. It is conceded that sea water contains magnesium in excess of that usually found in blood, and it is for that very reason it has proved useful in pointing the way for further research; but while it is found in the blood in the proportion of about two of magnesium to three or more of calcium (Hammarsten), the conditions are reversed in muscle and nerve, the proportion being about two of magnesium to one of calcium. One would therefore expect the quantity of magnesium to vary considerably at different times, and it is difficult to say what constitutes an excessive amount; an optimum has yet to be determined.

In conclusion it should be said that these results were to be expected in the light of those of Quinton,[1] who not only transfused dogs with isotonic sea water in quantities equal to the quantity of blood withdrawn, but also injected large quantities intravenously, without serious consequences. "Entre l'eau de

[1] Quinton, R., "L'Eau de Mer Milieu Organique," 1904, p. 160.

mer et le milieu vital du Vértebré, il y a physiologiquement identité."

Summary.

1. Strips of the ventricle of the turtle's heart will live as long in isotonic sea water as in Ringer's solution.

2. After "sodium chloride arrest," strips of ventricle will recover as well in isotonic sea water as in Ringer's solution.

3. The whole heart will beat as long in isotonic sea water as in Ringer's solution.

This problem was suggested to me by Professor Loeb, and I have to thank him for many helpful hints.

Note. — At the time these experiments were begun and the paper was in preparation, I was ignorant of the work of Mayer[1] on the embryo heart of the loggerhead turtle, which should be referred to.

[1] Mayer, Alfred G., "Rhythmical Pulsations in Scyphomedusæ," Carnegie Institution of Washington, Publication 47, 1906.

ON THE RELATIONSHIP OF THE FISHES OF THE FAMILY SIGANIDÆ.

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The relationship of the family Siganidæ has long been uncertain, though modern authors are unanimous in placing it near the family Acanthuridæ, which it resembles in form of body and in other external features. It was with the hope of finding some skeletal characters that might further indicate its relationship that this investigation was undertaken. The form chosen to represent its family was *Siganus fuscescens* from Japan.

DESCRIPTIVE.

The cranium does not depart very much in shape, nor with few exceptions the elements composing it very much in shape, size, or arrangement, from the percoid cranium with the superior ridges little developed as in the genus *Perca*. The myodome is well developed and opens widely to the exterior at the posterior end of the parasphenoid. The basisphenoid is absent, but at the front of the myodome in the usual place of the descending process from the basisphenoid the interorbital tissue is thickened and separates the eye muscles exactly as the basisphenoid process usually does. The alisphenoids are remote from each other. The exoccipitals meet on the superior surface of the basioccipital; the vagus foramen in the exoccipital is very large. The crest of the superoccipital is moderately developed, but does not reach to the anterior end of that bone. The frontals are large spongy bones, evenly convex transversely, and without ridges or canals. Posteriorly they reach to the epiotics at each side of the supraoccipital which is wedged in between them. To their anterior ends are attached the wide thin nasals, which are joined to each other at the median line and appear like a continuation of the frontals, arching widely over the nasal chambers forming a rounded roof open only outward and downward. The ethmoid is ossified some distance in front of the prefrontals, and is inter-

posed between the vomer and the nasal bones. Posteriorly it is continued backwards as a cartilaginous plate separating the prefrontals and behind them is connected with the tissue of the interorbital septum. The prefrontal is not pierced by the olfactory nerve, but the nerve runs to the nasal chamber through a passage

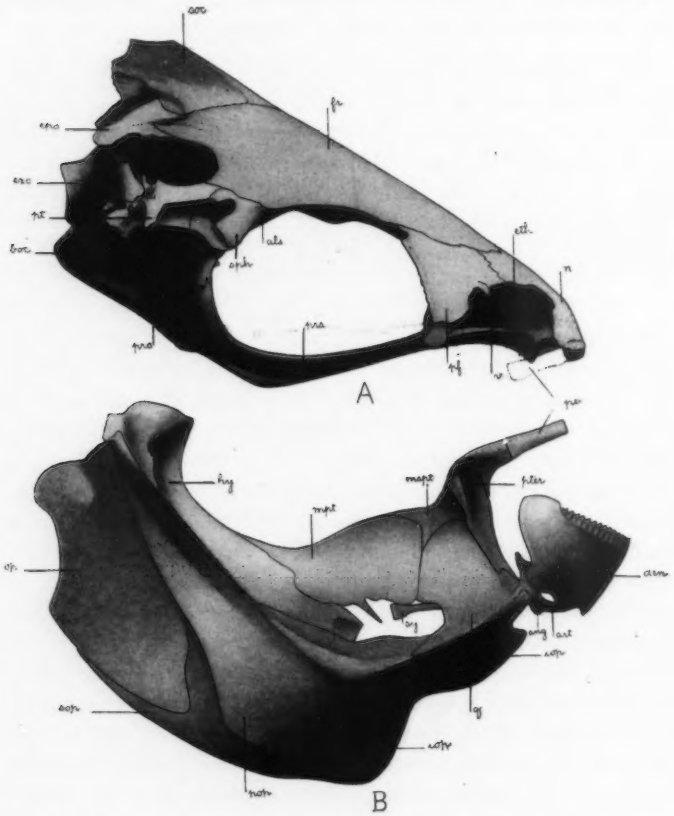


FIG. 1. *Siganus fuscescens*. *A*, lateral view of cranium; *B*, lateral bones o head. *als*, alisphenoid; *ang*, angular; *art*, articular; *boc*, basioccipital; *den*, dentary; *epo*, epiotic; *eth*, ethmoid; *exo*, exoccipital; *fr*, frontal; *hy*, hyomandibular; *iop*, interopercle; *n*, nasal; *mpt*, metapterygoid; *mspt*, mesopterygoid; *op*, opercle; *pa*, palatine; *pe*, prepalatine; *pf*, prefrontal; *pop*, preopercle; *pro*, proötic; *prs*, parasphenoid; *pt*, pterotic; *pter*, pterygoid; *q*, quadrate.; *soc*, supraoccipital; *sop*, subopercle; *sph*, sphenotic; *sy*, symplectic; *v*, vomer.

between the prefrontal and the ethmoid. The vomer is branched or Y-shaped and toothless. The opisthotic is present in its usual position covering the suture between the exoccipital and the pterotic. The parietal is entirely absent, and the frontal and epiotic bound the supraoccipital.[1] The remaining cranial elements are typical of the majority of the spiny-rayed fishes and are well shown in the accompanying drawings.

The post-temporal is forked and though the lower fork is very short it is continued by a ligament and joined to the opisthotic in the usual way so that the post-temporal stands away from the cranium as it does when the lower fork is long. The superior fork is rather broadly but not firmly joined to the epiotic. A couple of tunneled dermal bones, the supratemporals, are present on each side of the cranium in front of the post-temporal. The hypercoracoid foramen is large and is directly in the center of the bone. The actinosts are moderately long and somewhat constricted in the middle; three of them join the hypercoracoid and one the hypocoracoid. The upper pectoral ray works directly on the edge of the hypercoracoid. The postclavicle is a long, slender, curved ray of bone composed of an upper and a lower element. The other shoulder girdle elements are typically percoid in size, shape, and arrangement.

The lower part of the pelvic girdle extends forward as a long roughened plate just under the skin of the breast. From the upper surface of this plate the girdle is developed vertically upward, and meeting its opposite fellow at the upper edge, which is inclined towards it, incloses a chamber between. Anteriorly a long spine is sent forward from the upper edge of the girdle to between the clavicles. Backward over the base of the ventral fin a triangular spine is developed. The posterior or inner ventral fin spine is attached to the lower surface of this triangular pelvic spine — the anterior or outer ventral fin spine is attached to the base or posterior end of the breast plate. Some space is left between the ventral spines in which the three ventral rays are placed with their bases close together nearer the posterior ventral spine than the anterior.

[1] Several crania of different sizes (the smallest from a specimen 7 cm. in length) were prepared and examined on both the inner and outer surfaces, but no trace of the parietal was found.

The maxillary elements are of rather thin spongy bone rather solidly and immovably attached to each other though not anchylosed. The premaxillary carries a single row of bicuspid teeth (or tricuspid teeth if a scarcely developed third cusp be considered). The usual backward developed spine from the symphysis of the premaxillaries is scarcely developed, and instead of sliding over the vomer and ethmoid between the nasals, it abuts against the shallowly concave front of the ethmoid. A short process from each maxillary fits into a cup at the front of each arm of the vomer, and the movement of the upper jaw is a swinging motion from a hinge similar to that of the mandible rather than the usual sliding motion.

The opercular apparatus is complete and in no way peculiar. The preopercle has an extra long lower limb extending forward from its angle with the upper limb. The hyomandibular is long and with a simple unforked head. It sends no process to the metapterygoid, which occupies a position between the lower end of the hyomandibular and the quadrate. The mesopterygoid is very small but in the usual position between the metapterygoid and palatine and connected with the pterygoid below. The symplectic is small and slender and runs along the inner surface of the quadrate. The pterygoid and palatine are normal in arrangement, the latter attached to the lower edge of the prefrontal, but anterior to the palatine is a prepalatine bone. This is a cylindrical-shaped bone, suturally, but not immovably attached to the front of the palatine and extending anteriorly along the side of the vomer to the upper edge of the maxillary, very much as the anterior process on the palatine in the majority of spiny-rayed fishes does.[1]

The mandible is short and resembles the united maxillary bones in shape. The articular is very small and is almost covered from sight by the dentary. A small angular bone is present. The mandibular teeth are in a single row and similar to those on the premaxillary.

The suborbital chain is complete, but no suborbital shelf extends inward around the orbit.

[1] In order to make sure that the possession of this unique prepalatine element was normal three different specimens were examined.

There are two basibranchials; the hypobranchials of the fourth arch and the pharyngobranchials of the first are absent. There are three pharyngobranchials present on each side, each a thin concavo-convex plate, shaped like a clam shell, and bearing along its lower edge a single row of long, slender, comb-like teeth. A second, less complete row is situated towards the convex center of the plate, though on the first plate the second row is represented by a single tooth. Similar teeth are arranged in four or five "combs" placed obliquely across each slender lower pharyngeal. A third or more of the band of gill filaments of the first arch (and a decreasing portion of it on the succeeding arches), is free from the arch above its angle at the upper end of the ceratobranchial, and rises upward on a cartilaginous base at each side of the cranium in a cavity behind the eye.

The hyoid arch is in no way peculiar in form or arrangement of its elements, except that the paired hypohyals are larger than usual; a small glossohyal is present.

The vertebræ number as follows: thoracic 10 + caudal 12 + hypural = 23.

The first vertebra has a wing of thin lace-like bone developed outward and downward from the side of its neural arch to which the first epipleural is attached. The parapophyses are scarcely developed, but their small representatives are of about the same size on all of the vertebræ. The ribs are attached to the centræ of the vertebræ with the anterior edges of their bases fastened closely against the parapophyses. The epipleurals posterior to the first are attached to the ribs at some distance from the vertebræ. The spine bearing interspinous bones are somewhat wider than the ray bearing ones. They expand laterally at the bases of the spines, making a row of bony plates, which are evident through the skin of the entire fish. The hæmal, neural, interhæmal, and interneural all have a thin laminæ of bone developed backwards from their posterior edges. A long, strong process extends forward from the first interhæmal towards the pelvic girdle and forms a sharp abdominal ridge. A sharp spine projects forward from the first interneural at the base of the first dorsal spine and pierces the skin at the nape. The supplementary caudal rays are attached to the backward extending spinous processes of one or two vertebræ anterior to the hypural.

Relationships.

Dr. Gill in the "Standard Natural History" [1] places the family Siganidæ with the Acanthuridæ under the superfamily Teuthidoidea. The Teuthidoidea he believes to be descended from the Chætodontoid fishes while the plectognathous fishes are descended from the Teuthidoidea.

Dr. Jordan in his "Guide to the Study of Fishes" [2] follows the same arrangement but he places the families Acanthuridæ and Siganidæ together with the Chætodontidæ and other related forms in a large group, the Squamipinnes, though he considers the Acanthuridæ and the Siganidæ under different suborders, giving to the latter suborder the name Amphacanthi.

Either of these, with slight changes, is the order in which these fishes are arranged by all modern authors, and as it is apparently the most logical no other arrangement need be here referred to.

Though there is doubtless an alliance between the families Acanthuridæ and Siganidæ the alliance is certainly not close enough to place them in the same superfamily. The Teuthidoidea is defined by the "development of transverse, expanded, buckler-like, subcutaneous plates on the back intervening between the spines, and limiting their erection forward."

The expanded interspinous rays that form the bony bucklers at the base of the spines are developed in this respect only to a slightly greater degree than may be found in many scombroid fishes, and not to so great a degree as in some berycoid fishes.

The fishes of the Acanthuridæ [3] differ from those of the Siganidæ in having the cranium wedge-shaped or tapering to a point at the ethmoid region, the parasphenoid drawn out downward into a wide, thin, sharp plate before the orbital region; the post-orbital part of the cranium shortened; the parietal present; the post-temporal suturally attached to the cranium and forming an integral part of it (this true of *Hepatus* to a greater extent than of

[1] Cassino & Co., Boston, 1885, Vol. III.

[2] Henry Holt & Co., New York, 1905, Vol. II.

[3] *Hepatus bahianus* and *Xesurus punctatus* represent the family Acanthuridæ in this investigation.

Xesurus)[1] the parapophyses developed on all the vertebræ; the postclavical composed of a single piece, though of a similar long, slender shape to that of *Siganus*.

In spite of these differences *Siganus* appears to be more closely related to the family Acanthuridæ than to any other known group. It resembles the fishes of the family Acanthuridæ in having the maxillary and mandibular elements short and resembling each other in shape; the maximillary and premaxillary solidly attached to each other; the spines from the symphysis of the latter short; and the movement of the maxillaries similar to that of the mandible; the teeth flattened, in a single row, and without entire edges; the suborbitals without a shelf below the eye-ball; the gill-filaments extending free above the gill arches in a cavity at each side of the cranium; a spine developed forward from the first interneural spine; the gill openings restricted to the sides.

In the connection of the maxillary elements to the cranium *Siganus* resembles *Balistes* and the other plectognathous fishes, in which the maxillary elements fit against the concave front of the ethmoid region and have a hinge-like movement without any sliding motion forward. In the acanthuroid fishes the ethmoid region is hemispherical in front, and the maxillary elements fit socket-like over it, with very short premaxillary spines above. A movement similar to that in *Siganus* is produced by the maxillary elements turning on the ethmoid ball so that the premaxillary spines glide over the ethmoid somewhat as in the majority of fishes, but there is no sliding motion straight forward.

In most characters, however, the acanthuroid fishes resemble *Balistes* more closely than does *Siganus*. The shape of the cranium is strikingly similar: tapering forward; the parasphenoid extending down in a wide thin plate; the postorbital region shortened; the sphenotic and prefrontal regions curving around the eye; and the postclavicle a long simple ray of bone.

In all of the plectognathous fishes the premaxillary is firmly

[1] The attachment of the post-temporal to the cranium apparently has not the importance sometimes given to it as Dr. Gill has shown in his paper on the affinities of the Ephippiids (Proc. U. S. Nat. Mus., Vol. V., p. 557), or as the present writer has shown in his work on the shoulder girdle of the Hemabranchiate fishes (Proc. U. S. Nat. Mus., Vol. XXV., p. 619).

anchylosed to the maxillary. In the Acanthuridæ and Siganidæ these elements are immovably attached to each other but are held together only by connective tissue.

The peculiar pelvic girdle of *Siganus* has its counterpart in the berycoid fishes, but in this connection probably means nothing. The modification of the girdle is brought about by a development upward of the middle portion so that a chamber is inclosed between the opposing sides.

Summary.

Siganus stands rather widely away from any known form. The possession of the peculiar prepalatine element and the two spines to each ventral preclude a close relationship to any living fishes. Though its relationship to the acanthuroid fishes is not close it apparently was descended from some form near that stock, and the condition of the maxillary elements, particularly their attachment to the cranium, indicate a relationship in the plectognathous direction rather than in the Chætodontoid.

If this be true *Siganus* can only be an off-shoot at one side from some acanthuroid form having the plectognathous articulation of the upper jaw to the cranium. The acanthuroid fishes are in a more direct line with the plectognathous fishes and *Siganus* could not stand between them.

The plectognathous fishes show degeneration from the acanthuroid stock by a series of continuous and ever increasing steps. *Siganus*, on the contrary, shows development in the direction of higher specialization.

The characters of *Siganus* are apparently of sufficient value to entitle it to independent superfamily rank at least, or to a rank coördinate with that of the acanthuroid fishes.

THE FERTILIZATION OF AMŒBA PROTEUS.

GARY N. CALKINS.

Three years ago I published a short paper entitled "Evidences of a Sexual Cycle in the Life-history of *Amœba proteus*"[1] in which I described the formation of chromidium in one life-phase of this common rhizopod and the subsequent formation of secondary nuclei. The latter were interpreted as nuclei of the supposed gametes which were collected in a cyst. The supposed gametes were in no case seen to emerge from the cyst and conjugation was not observed. Many of the structures observed in the amœbæ at this time, could not be interpreted in terms of the corresponding phases of other fresh water rhizopods, a curious division of the granules (represented in Plate 3, Fig. 23 of the former paper), and an equally enigmatical series of spheres with peripheral granules (represented in Figs. 12, 24 and 27), being particularly hard to homologize with other known phases in rhizopod development. Had I not been busy with other work at the time, I might have discovered that the very material used for these "evidences" would furnish proof of the actual fertilization, for this last spring, hoping to find some trace of a maturation process in rhizopods at the period of chromidium formation, I dissolved off the cover glasses from the amœbæ which were preserved in balsam, embedded them in paraffine, cut them one by one in sections from three to five microns in thickness and discovered the method of fertilization. A careful examination of the so-called "dividing granules" in these sections revealed the fact that what I had interpreted in the total mounts as dividing forms of the chromidial granules, were actually minute nuclei in the process of fusion, and that, instead of division, it was the process of fertilization, while the encysted bodies with the peripheral granules were stages in the development of these fertilized nuclei. The material, unfortunately, is still not complete enough to give the details of the chromatin changes as thoroughly as I wish, but there are enough stages to enable us to clear up this sexual phase

[1] *Arch. f. Protistenk.*, V., 1904, pp. 1–16.

in the cycle of *Amœba proteus* and to bring together the observation of Scheel and earlier observers and to combine them in one completed life history.

As described in my earlier paper, this phase of the life-history of *Amœba proteus* is characterized by the repeated division of the nucleus until many nuclei are present in the cell, 72 to 80 being the largest numbers observed in any one organism. These nuclei then fragment, and the chromatin granules, liberated by rupture of the nuclear membranes, are distributed in the cytoplasm. This fragmentation continues until all but one of these primary nuclei are thus broken up, this one remaining as a residual nucleus, while the cytoplasm becomes packed with the chromatin fragments which I had interpreted as the chromidium. Stages in this disintegration of the nuclei are shown in Figs. 6, 7, 8, 19–26, of my earlier paper. These granules were described as increasing in size, dividing and ultimately forming the hollow spheres with peripheral granules in the encysted stage (Figs. 23, 24 and 27 of the former paper).

Now that the amœbæ have been removed from the slides, sectioned, and stained in iron-hæmatoxylin, the structure of the granules is brought out with more vivid clearness than in the total preparations stained with picro-carmine. The disintegration of the primary nucleus can be followed step by step in the sections and the origin of the gametic nuclei, as I may now call them, can be easily traced.

The first indication of fragmentation is the collection of the chromatin about the inner walls of the primary nucleus. Comparatively large reservoirs are massed about the periphery in this way (Fig. 1), but in the meantime chromatin granules in the interior of the nuclei are assuming a definite form, while a less deeply staining, more homogeneous cortical zone of nuclear plasm collects around them. The peripheral granules are also used to form similar minute nuclei which, apparently as soon as formed, move out into the cytoplasm. Here they are clearly marked nuclei, consisting of a densely staining central granule or karyosome with a more faintly staining cortical zone (Figs. 2, 3, 4, 5, 6, 7). The bulk of the primary nuclei is metamorphosed into these secondary nuclei, which are so small and so numerous that they give a characteristic granular appearance to the cell.

The earliest stages of secondary nucleus formation within the nucleus are so minute that they would scarcely be taken for the same things as the cytoplasmic nuclei. Stages in growth, however, can be found in which the size varies from these extremely minute ones to the full size nuclei of the cytoplasm (Figs. 1, 2). Fig. 5 shows a primary nucleus in the process of fragmentation with two full size secondary nuclei emerging at *a* while within the nucleus one or more large ones can be made out. Figs. 6 and 7 and Fig. 8 show similar late stages in secondary nucleus formation.

After emerging from the primary nucleus the secondary or gametic nuclei fuse and the stages in the process can be followed step by step in the fixed material. In such fixed material, however, the argument may be raised that it is equally possible to trace the history of stages in the opposite direction and claim that the process is one of division and not conjugation, this being one of the serious difficulties in working on preserved organisms. Nevertheless the evidence is so strong that there exists no doubt whatsoever in my own mind that we are dealing with conjugation and not with division. In the first place the number of gametic nuclei is far greater than the number of sporoblasts which make up the later cyst stage. In one specimen I counted more than three hundred gametic nuclei in addition to eighteen as yet unfragmented primary nuclei, while the number of sporoblasts in the later stages does not exceed 250 in any one of the specimens in my possession. Numerical relations indicate, therefore, that union rather than division takes place. In the second place the individuals of the pairs of nuclei that are fusing, are of the same size as the single ones. If they were dividing the daughter nuclei would be considerably smaller. Size here, however, is so variable that I do not lay much stress on this argument. In the third place, if the nuclei were dividing we should find dumb-bell shaped figures with the diameter of the nuclei drawn out at right angles to the plane of division. This is not the case, the minute nuclei remaining as spherical as though not in contact. In the fourth place we should expect to find connecting strands of chromatin substance between the recently divided karyosomes if it were a case of division, but no such connecting strands exist. In

the fifth place we should expect to find the daughter karyosomes elongated in the axis at right angles to the plane of division if it were division. Such is not the case as inspection of the figures shows, while in many cases the two karyosomes are elongated in an opposite direction (Fig 2, *d*; Fig. 3, *c*). In the sixth place if it were division we should expect it to take place more rapidly than the figures indicate, for in fusion, the process in protozoa requires a longer time than does division and the large number of double forms of these secondary nuclei indicates that the process is a relatively slow one.

On the whole, therefore, I believe the evidence justifies no other conclusion than that this is a process of fusion and not of division and that we are dealing here with an actual conjugation of nuclei. The fusion takes place by preliminary union of the extreme peripheries, this is followed by union of the homogeneous portions, and finally by union of the karyosomes (Figs. 2, 3). In one or two cases I have seen some evidence that more than two nuclei may thus fuse. Fig. 2, *e*, for example presents such a case, the larger size, and the two karyosomes indicating that fusion of two nuclei has already taken place. It occurred to me that the minute nuclei, before fusing, might possibly divide in some form of maturation division, but I have been unable to confirm this supposition in the material at hand, and incline to the belief that fusion occurs at once, for the uniting nuclei are abundant in the immediate vicinity of disintegrating primary nuclei.

The result of this fusion of gametic nuclei is, in each case, a nucleus of somewhat larger size in which the central granule fragments into a cloud of extremely minute chromatin granules lying about a central space which is the beginning of the vacuole characteristic of the later phases in development of the spores (Figs. 9, 10, 14). These granules next collect in small aggregates which are arranged about the periphery of the vacuolated mass, from 70 to 100 of them, as nearly as I can estimate, being formed in each of the many centers which now correspond to those multiplication centers of sporozoa called sporoblasts by Schaudinn. In this period, the sporulating centers or sporoblasts, are carried about in the cytoplasmic flow and appear as small

hollow nuclei which, in many cases, resemble the nuclei of some *Pelomyxa*-like forms. In the specimen from which Fig. 11 of my original paper was taken, there are more than 200 of these centers of multiplication, while in the encysted form shown in my original Fig. 12, a section of which is shown here in Figs. 13 and 14, there are about 250 of these sporoblasts.

The ultimate form assumed by the *Amœba* in the material which I possess, may be described as a collection of these sporoblasts, each one of which is a hollow sphere, the walls being studded with minute granular nuclei from 70 to 100 in number (Fig. 14). They lie about the one remaining primary nucleus which is shown in the section reproduced in Fig. 13.

A first examination of these nuclei in the sections gives the impression that the amœba body is well infested by parasites and this indeed, was my belief until a critical examination of the material in all stages. convinced me of my error. While under the belief that these nuclei were parasites I sought to interpret the several phases of the *Amœba* cycle which was described in 1904, as effects of such an infection. I concluded that minute parasites enter the body of *Amœba*, stimulate the nucleus to divide as does *Plasmodiophora brassicæ* the cell nuclei in the cabbage root, and then multiply by division in the interior of the endoplasm, the rapid multiplication filling the body with the minute granules which, earlier, I had interpreted as chromidium granules. My impression was strengthened by the observations of Schubotz[1] who interpreted the nuclei which I had described in *A. proteus*, as degenerating nuclei, an interpretation with which I quite agree, although not in the sense he meant. Prandtl[2] still more recently has published an interesting account of the development of young forms of *Allogromia* as parasites in the endoplasm of *Amœba proteus* and he also agrees with Schubotz that the nuclei described in my earlier paper are degenerating nuclei, and suggests that my "chromidium granules" may be young phases of an organism similar to the *Allogromia* which he describes.

While frankly admitting the possibility that these small nuclei may be parasites, a possibility which with fixed material and on

[1] "Beiträge zur Kenntnis der *Amœba blattæ* und *A. proteus*," *Arch. f. Prot.*, VI., 1905.

[2] "Der Entwicklungskreis von *Allogromia* sp.," *Arch. f. Prot.*, IX., 1907.

morphological grounds alone, cannot be entirely refuted, I firmly believe that they are not parasites but developmental phases of *Amœba proteus*. My reasons for this belief may be briefly summarized as follows: First, the early stages of nuclear increase are present in specimens of *Amœba* in which there are none of the supposed parasites. Second, the supposed parasites must originate inside of the supposed degenerating nuclei, for, as Figs. 2, 5, 6, 7 and 8 clearly show, the structures under consideration first arise inside of the nuclei, and wander outside by dissolution of the nuclear membrane. Third, if they are parasites they must conjugate inside of the endoplasm of the *Amœba*, for, as we have seen above, there are no good grounds for interpreting these structures as dividing forms. Fourth if the structures in question are conjugating parasites the conjugation leads to further development within the protoplasm of the host cell without any protecting membranes against the resistance of the host cell. Fifth, if these things are parasites, then the secondary nuclei of *Arcella*, *Polystomella* and *Entamœba* must likewise be parasites, for the resemblance between the several cases is too strong to allow another interpretation. Finally, if they are parasites, they must wander into the nuclei of *Amœba proteus* in the form of germs too small to be recognized and grow there into larger nucleus-like bodies which emerge from the nucleus and conjugate, and all this without disturbing the physiological equilibrium of the *Amœba*, and without effecting any pathological change such as formation of vacuoles or spaces about themselves (cf. Figs. 2, 3, 4). The evidence, therefore, is altogether in favor of the nuclear character of these questionable structures, and their union, I believe, can be interpreted in no other way than as the fertilization of this universal rhizopod.

The fertilization of *Amœba proteus* has been sought for by biologists for decades. Many observations have been published on phenomena supposed to be conjugation processes, but these in the main, have turned out to be cases of plastogamy, common amongst the rhizopods, or cases of engulfing of one by another. If the fertilization were any ordinary process similar to what occurs in other allied forms, there seems little likelihood that it would have been overlooked for these many years. But occurring

as I have now shown it to occur, in a manner quite unlike that of the majority of other rhizopods that we know, the reason for its being overlooked becomes apparent. Many have observed *Amœba proteus* in the multinucleate condition. Carter in 1863 observed as many as 70 nuclei in specimens of *Amœba princeps* which is usually regarded as the same as our *Amœba proteus*, and Wallich in the same year observed the liberation of many fine granular bodies by the rupture of the nuclear membrane, while Schaudinn[1] calls attention to the fact that he has observed the nuclear multiplication and suggests that it betokens a possible sexual phase.[2]

The process of fertilization, as I have described it here, comes under the head of the conjugation phenomena known as endogamy, or conjugation of nuclei within the original cell parent. It is not the only instance of such a phenomenon amongst the *Sarcodina*. Hertwig, in 1898, described self-fertilization in the case of *Actinosphærium*, while Schaudinn in 1903 (*loc. cit.*) described it in the case of *Entamœba coli*, the harmless commensal of the intestine. In both cases, however, the process is described as much more complicated than that which I have outlined here. In *Actinosphærium* the vegetative nuclei are reduced by fusion or by absorption to a relatively small number. The cell then divides into as many daughter cells as there are nuclei (five to ten); these daughter cells encyst within the parent cyst, the nucleus divides by mitosis and each of the cysts divides into two daughter cysts, each with one nucleus. The nucleus in each of these daughter cysts next divides twice, giving rise to two "polar body" equivalents. The cytoplasm and remaining nuclei of the two daughter cysts then fuse and the fertilization is completed by the reunion of the parts. In *Entamœba* the process is more like that of *Amœba* as described here. The cell throws out foreign matter and waste products of its own metabolism, and becomes smaller, more compact and more spherical. After secreting a gelatinous membrane in which the cell remains encysted, the cell nucleus divides into two nuclei, the division being followed by an incom-

[1] "Untersuchungen über die Fortpflanzung einiger Rhizopoden," *Arb. a. d. Kais. Gesundh.*, XIX., 1903.

[2] For further historical data see my original paper.

plete division of the cell body. After this division, the daughter nuclei fragment, forming a mass of minute chromidial granules which are distributed throughout the cell. From the two masses of granules thus formed secondary nuclei arise by fusion as in the case of *Centropyxis*, *Arcella*, *Polystomella*, and rhizopods generally, and these two nuclei, after preliminary division giving rise to what Schaudinn interprets as "polar body" equivalents, divide for a third time, the products of this division fusing two by two. The partly separated protoplasmic body then reunites and the fertilization cell contains two fertilization nuclei. These two nuclei then divide twice forming eight nuclei altogether and these finally become the nuclei of eight amœboid spores.

Except for the maturation divisions, which future study may reveal, the process of fertilization in *Amœba proteus* is thus strikingly similar to that of *Entamœba coli* the gametic nuclei arising by fragmentation instead of by division as in the latter case. The fertilization nucleus forms, not eight, but many daughter nuclei, and from analogy, I would expect these vacuolated centers of multiplication or sporoblasts in *Amœba proteus* to produce from 70 to 100 pseudopodiospores.

The formation of the secondary nuclei in *Amœba proteus* differs in some important respects from the process in other rhizopods. In *Arcella*, *Centropyxis* and *Polystomella* for example, it occurs in the chromatin substance that is either transfused through the membrane of the nucleus or formed by fragmentation of the nuclei. In all cases that have been worked out, however, the secondary nuclei are formed from the substance of this chromidium and we can thus trace their history back indirectly to the primary nuclei. In *Amœba proteus* on the other hand, the nuclei are not formed from diffused chromatin nor from the fragments of primary nuclei but they form directly within the primary nuclei and emerge from it as fully formed secondary nuclei. In this case, as in the other cases, the secondary nuclei are the gametic nuclei, only here their union is, so to speak, precocious and without the customary gamete formation, while the union and especially the further development *within the parent cell*, are unusual and unexpected discoveries.

Scheel takes up the life history of *Amœba proteus* from this

stage on. In his paper on the Encystment of *Amœba proteus*[1] he describes compound cysts which I would interpret as a late stage (with protecting gelatinous membranes), of the stage shown in my Fig. 12 of the original paper (section shown in Figs. 13, 14 of present paper). Each one of his cysts I would interpret as one of the sporoblasts (Fig. 14), which, by independent growth, reaches the size he describes (about 80 microns). The peripheral granules (Fig. 14, *b*) of my sporoblasts become his nuclei of the cyst and later, the nuclei of the minute amœbæ with stellate pseudopodia (*Amœba radiosa*).

In conclusion I would substitute for the tentative life-cycle published three years ago, the following: The ordinary *Amœba proteus* reproduces asexually by division (seen in every laboratory); ultimately the asexual cycle is replaced by the sexual, the conditions of which, periods, etc., are entirely unknown; the sexual cycle is inaugurated by the multiplication through division of the nuclei until many "primary" nuclei are formed; these primary nuclei fragment directly into minute granular nuclei corresponding to the "secondary" nuclei of *Polystomella*, *Centropyxis*, etc. In *Amœba* the secondary nuclei may be called the "gametic" nuclei; the gametic nuclei fuse to form fertilization nuclei; in these the fused karyosomes fragment to form finely divided chromatin (it is, strictly speaking, not a chromidium for it is entirely intra-nuclear), while a vacuole forms in the interior; this vacuolated fertilization nucleus becomes a center of multiplication (equivalent in every way to a sporozoön sporoblast); by accumulation of these fine chromatin granules the peripheral or "tertiary" nuclei are formed; the tertiary nuclei, surrounded by a minute bit of plasm, grow into the pseudopodiospores observed by Scheel (hypothetical); these young pseudopodiospores break away from the parent cyst and develop into young amœbæ formerly known as *Amœba radiosa*, and these, in turn, develop into the ordinary *Amœba proteus* of pond and laboratory.

COLUMBIA UNIVERSITY,
NEW YORK CITY, August 10, 1907.

[1] Festschrift für Carl von Kupffer, Jena, 1899.

Explanation of Plate XI.

The photographs are from sections, stained with iron-hæmatoxylin, of the same specimens of *Amœba proteus* that were pictured in my previous paper on "Evidences of a Sexual-cycle in the Life-history of *Amœba proteus*. The magnifications vary from 550 diameters (Fig. 13) to 2,000 diameters (Figs. 9 and 10).[1]

Fig. 1. Part of section of *Amœba* with about 30 primary nuclei some of which have begun to fragment. The chromatin is massed in a characteristic manner about the periphery while the small points in the center are the karyosomes of the future secondary nuclei. At this stage there are few secondary nuclei in the cytoplasm. × 1,000.

Fig. 2. Section from the amœba represented in Fig. 7 of my previous paper. The majority of the primary nuclei have fragmented and the cell body is spotted with the secondary nuclei. These may be seen *forming* in the large primary nucleus (*f*). The photograph also shows many stages in the fusion of the secondary nuclei. At (*a*) two are seen with the peripheries in contact; at (*b*) the bodies are beginning to fuse; at (*c*) and (*d*) fusion of bodies is completed, while the karyosomes have not yet fused but lie facing one another (if this were division the karyosomes would be elongated in the direction at right angles to this); at (*e*) there is an apparent double fertilization; at (*g*) the karyosome from the upper nucleus has migrated into the lower nucleus before fusion of the nuclei is complete. × 1,500.

Fig. 3. Primary nucleus with brood of young secondary nuclei within it and several secondary nuclei in various stages of fusion (*a*), (*b*) and (*c*) representing different stages in the process. × 1,350.

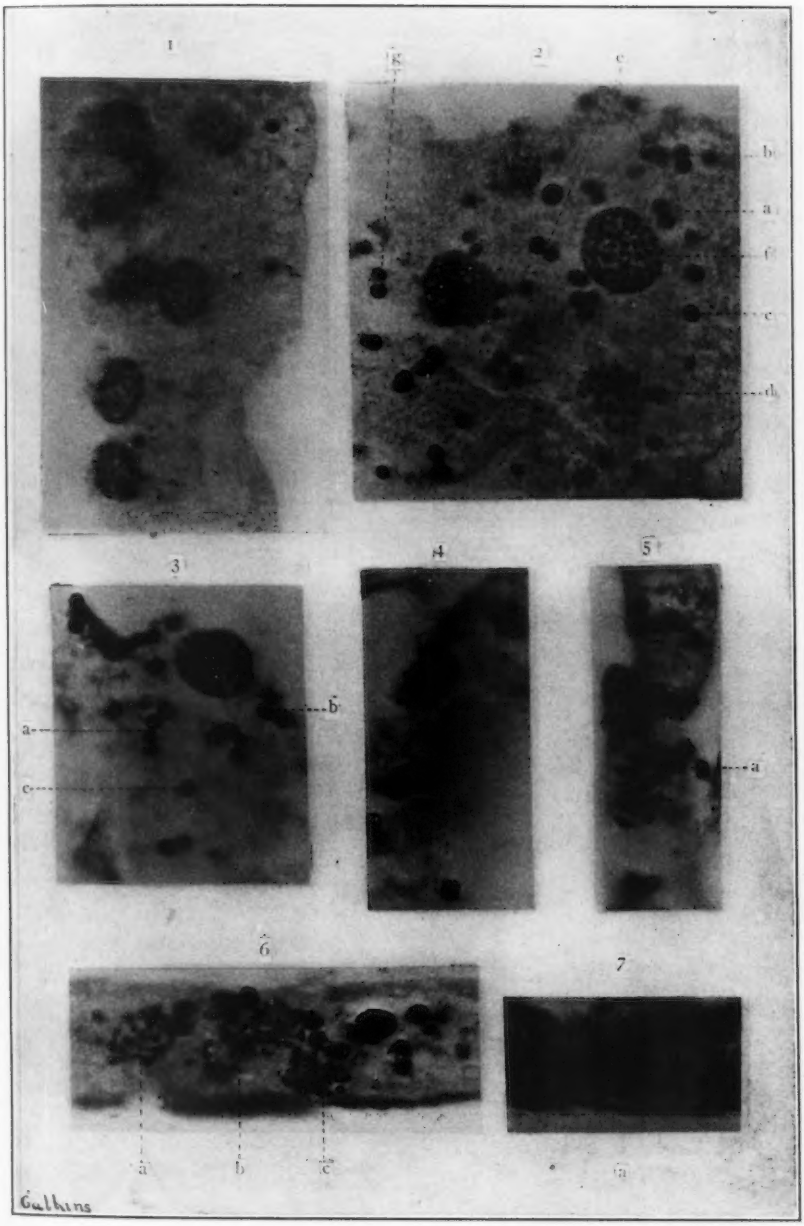
Fig. 4. Primary nucleus with most of the secondary nuclei gone. Some secondary nuclei below in different stages of early fusion, while above are two later stages of union. × 1,500.

Fig. 5. Primary nucleus in the process of liberating secondary nuclei. Two fully formed secondary nuclei are passing into the cytoplasm at (*a*), while others not yet formed remain in the nucleus. Fig. 7 is a deeper section of the same primary nucleus showing the fully formed secondary nuclei within it. × 1,500.

Fig. 6. Section of an amœba in which all the primary nuclei (*a*), (*b*), (*c*) are fragmenting and disintegrating while the secondary nuclei are in various stages of fusion. The section was injured by the objective so that some of the pairs of secondary nuclei are spread. × 1,500.

Fig. 7. Section of same nucleus as that shown in Fig. 5, showing brood (*a*) of secondary nuclei not yet liberated. × 1,500.

[1] The actual dimensions in the photographs must be increased by one fifth to give the magnifications stated.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EXPLANATION OF PLATE XII.

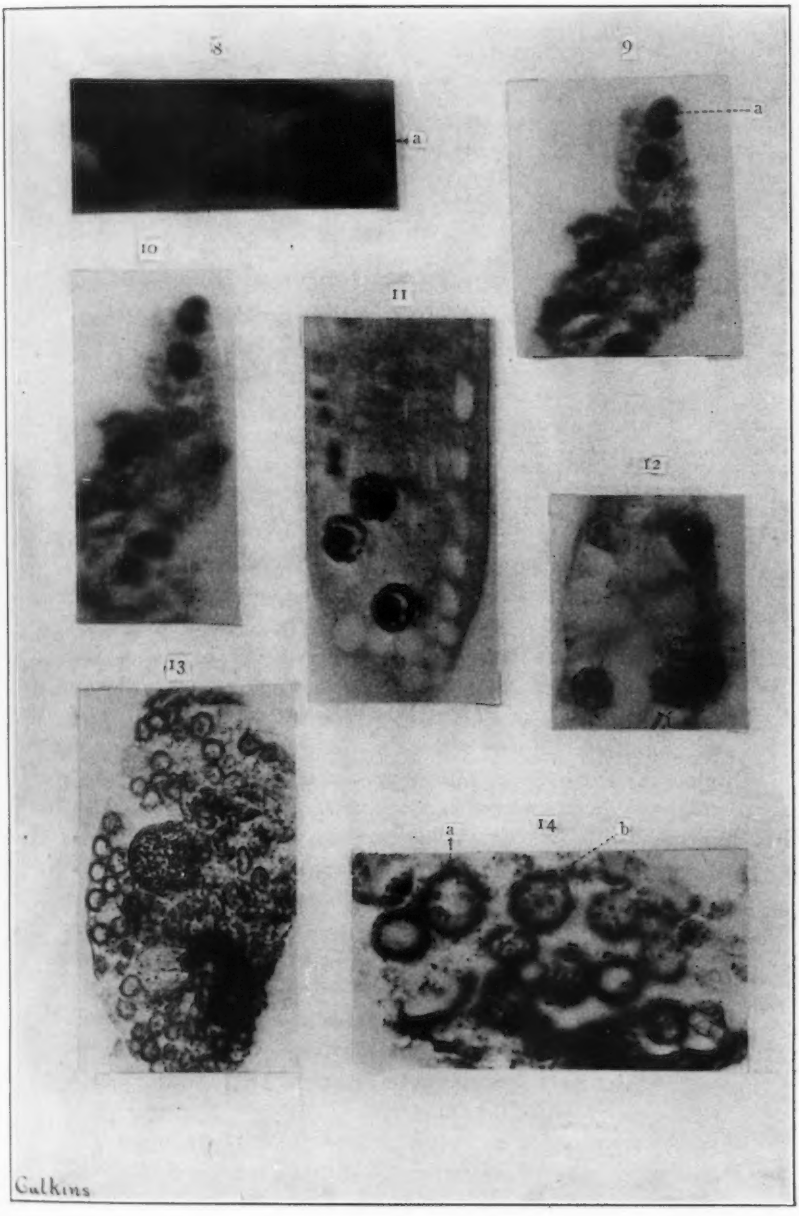
FIG. 8. Two primary nuclei showing broods of secondary nuclei (*a*).

FIGS. 9 and 10. Development of the fertilized nuclei, two photographs of the same section at slightly different foci. The upper nucleus at (*a*) shows the characteristic vacuole which becomes the vacuole of the later stages (cf. Figs. 13 and 14). The karyosomes fragment into minute chromatin granules which can be seen in Fig. 9. × 2,000.

FIG. 11. Later stages in development of the fertilized nuclei; the chromatin granules from the disintegrated karyosome now form accumulations about the periphery, these, later, form the nuclei of the spores. × 1,400.

FIG. 12. Intermediate stages in development between that shown in Fig. 9 and that of Fig. 11.

FIGS. 13 and 14. Sections of the amœba pictured in Fig. 12 of my earlier paper in the stage of encystment. In Fig. 13 (× 550) the primary nucleus shown is the residual nucleus comparable to the primary residual nucleus in the case of *Polystomella*. Here it is surrounded by many sporoblasts, which, in Fig. 14, are shown more highly magnified (× 1,500). At (*a*) and (*b*) the small but perfect peripheral nuclei may be clearly seen.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a
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a
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Calkins